Supplementary Information for:

Influence of size and charge of unstructured polypeptides on pharmacokinetics and biodistribution of targeted fusion proteins

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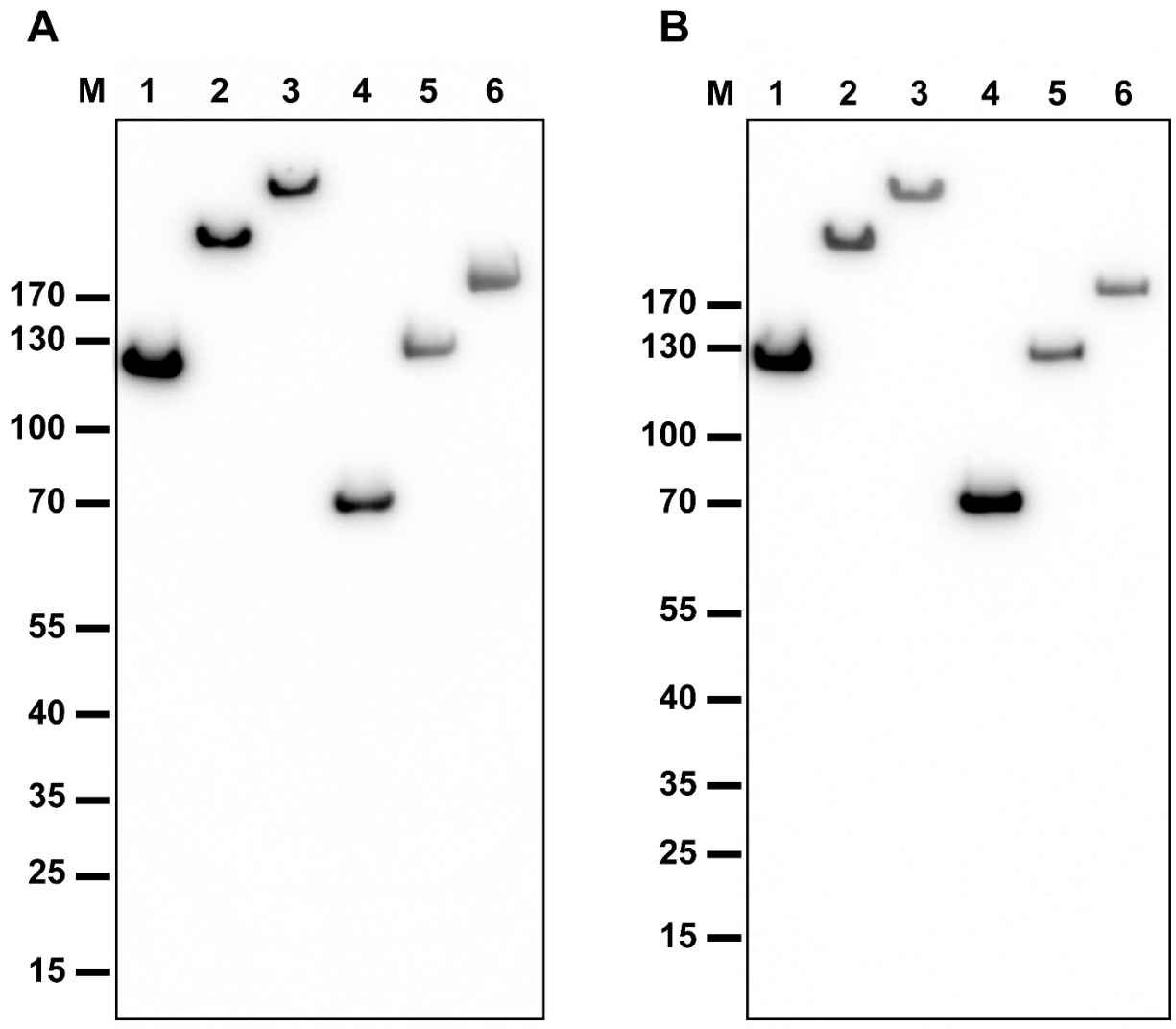


Figure S1: *Detection of N- and C-terminal tags of polypeptide-DARPin fusion proteins for assessment of purity by Western blotting and chemiluminescent immunodetection. Fusion proteins (13 pmol) were separated on a 4-12% Bis-Tris NuPAGE Novex SDS-PAGE and blotted to a PVDF membrane. Detection of the N-terminal FLAG-tag (A) and the C-terminal* His_6*-tag (B) with a mouse anti-FLAG M2 and anti-*His_4 *primary antibody. The recorded chemiluminescence signals were generated by substrate conversion of a goat anti-mouse IgG HRP conjugate. 1) PAS300-Ec1, 2) PAS600-Ec1, 3) PAS600-Ec1, 4) XTEN288-Ec1, 5) XTEN576-Ec1, 6) XTEN864-Ec1.*

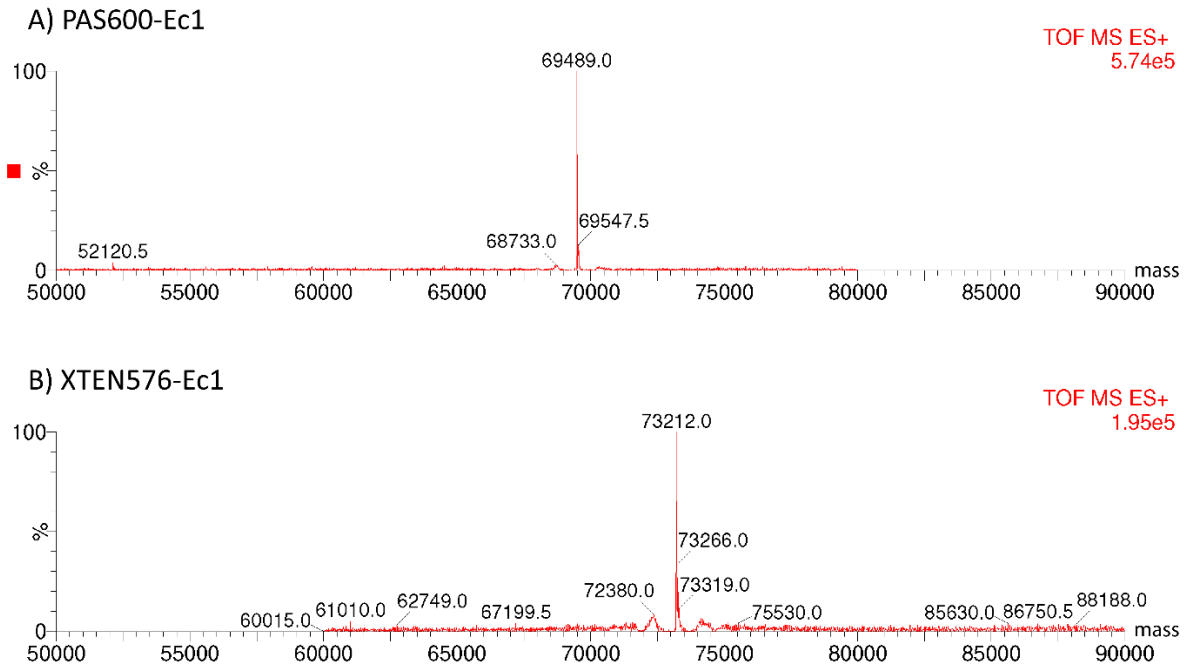
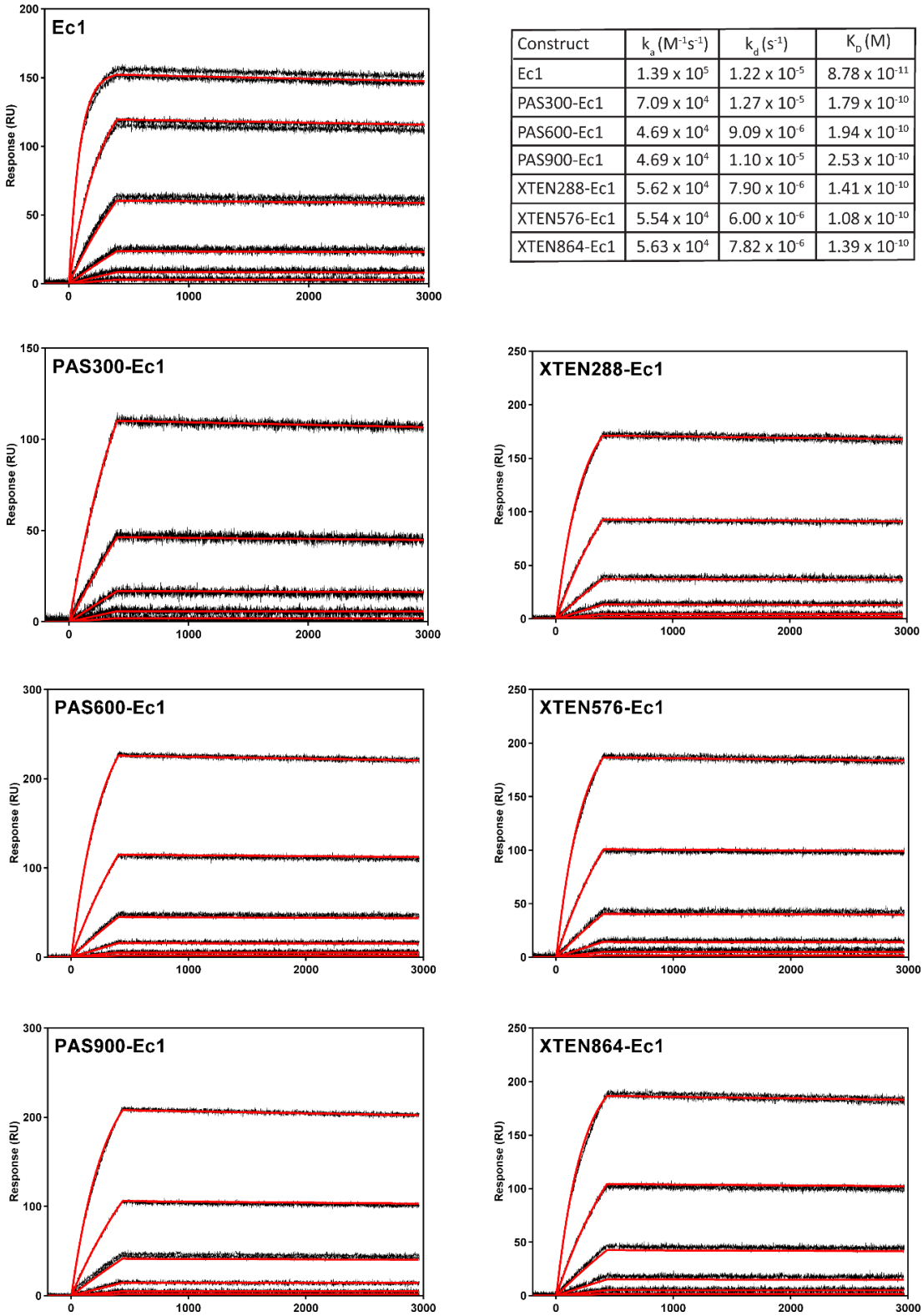


Figure S2: *Representative ESI-MS of PAS600-Ec1 and XTEN576-Ec1 showing monodisperse protein preparations as single peaks. The samples were analyzed in a mass range between 50 and 5000 Da, the sampling cone energy was set to 35 V. The m/z data were deconvoluted into MS-data using MaxEnt1 Software with a resolution of the output mass 0.5 Da/channel and Uniform Gaussian Damage Model at half height of 0.7 Da. All samples were measured within the expected mass range with 0.1 % accuracy.*



Construct	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
Ec1	1.39×10^{5}	1.22×10^{-5}	8.78×10^{-11}
PAS300-Ec1	7.09×10^{4}	1.27×10^{-5}	1.79×10^{-10}
PAS600-Ec1	4.69×10^{4}	9.09×10^{-6}	1.94×10^{-10}
PAS900-Ec1	4.69×10^{4}	1.10×10^{-5}	2.53×10^{-10}
XTEN288-Ec1	5.62×10^{4}	7.90×10^{-6}	1.41×10^{-10}
XTEN576-Ec1	5.54×10^{4}	6.00×10^{-6}	1.08×10^{-10}
XTEN864-Ec1	5.63×10^{4}	7.82×10^{-6}	1.39×10^{-10}

Figure S3: *SPR analysis of the binding kinetics of polypeptide-DARPin fusion proteins and unmodified DARPin Ec1 to EpCAM. For all measurements, the biotinylated extracellular domain of human EpCAM (biohEpEx) was immobilized to give 500 RU. Serial dilutions (0.33, 1, 3, 9, 27, 81 nM) of the proteins were injected at a flow rate of 60 µL/min. The ligand surface was regenerated by short pulses of 100 mM phosphoric acid. The resulting sensorgrams were fitted with a 1:1 Langmuir model and kinetic parameters k_a, k_d and K_D were determined for each interaction. Replicate injections are shown in black, fits are shown in red.*

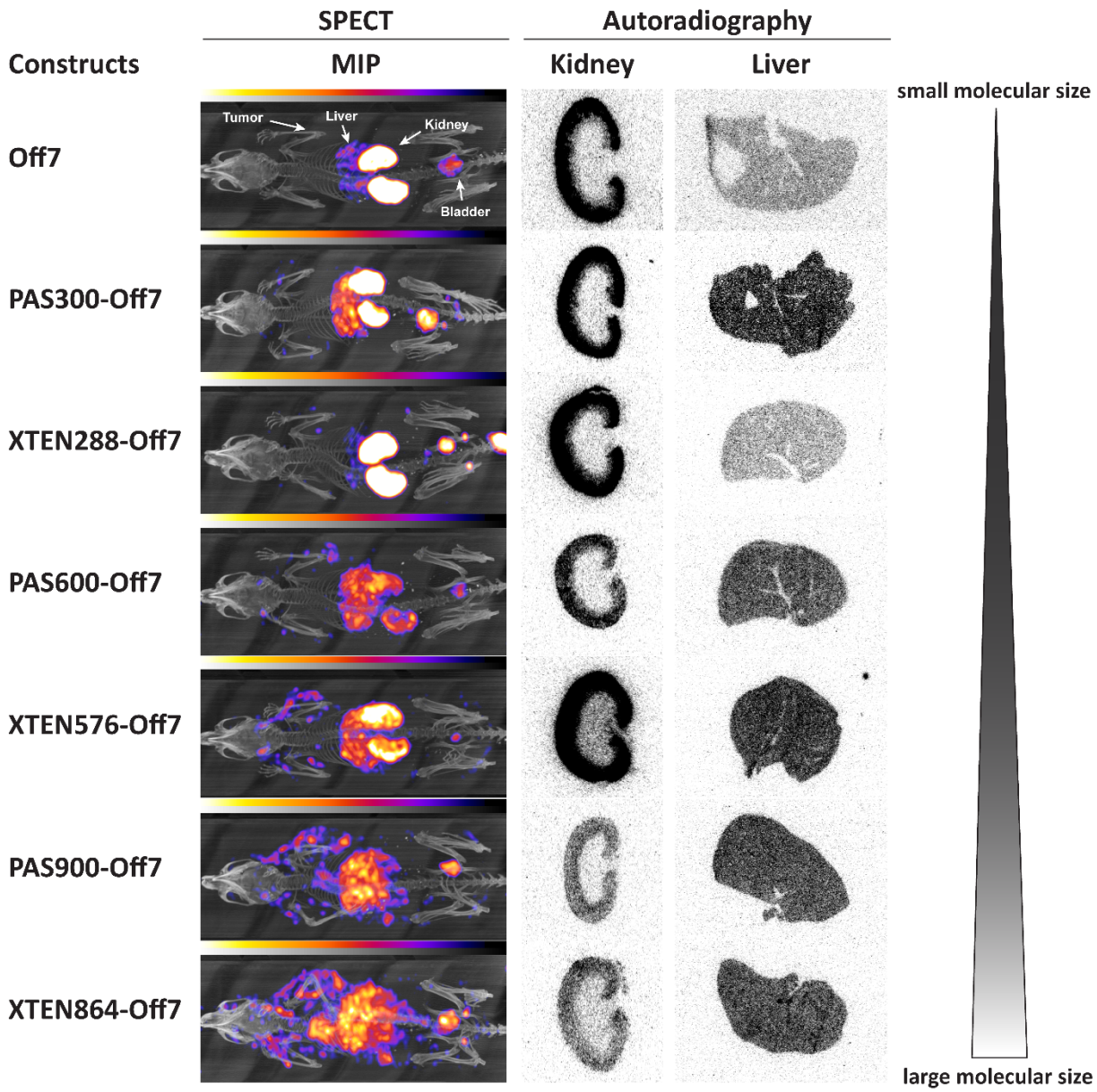


Figure S4: *Whole-mouse single-photon emission computer tomography (SPECT) imaging and tissue autoradiography of kidney and liver from mice with EpCAM-positive HT29 tumor xenografts with a non-binding control DARPin. Mice were injected i.v. with 532 pmol [^{99m}Tc](CO)$_3$-labeled control polypeptide-DARPin Off7 fusion protein or unmodified control Off7 with 7.5 MBq specific activity. Twenty-four hours after injection one mouse was anesthetized for SPECT imaging with isoflurane and imaged for 2 h. SPECT-images correspond to the maximum intensity projection. For kidney and liver autoradiography, organs were frozen in tissue embedding solution ex vivo and cut into 5 µm thick slices using a cryostat. The slices were placed on phosphor imaging plates overnight and imaged using a phosphor plate reader.*

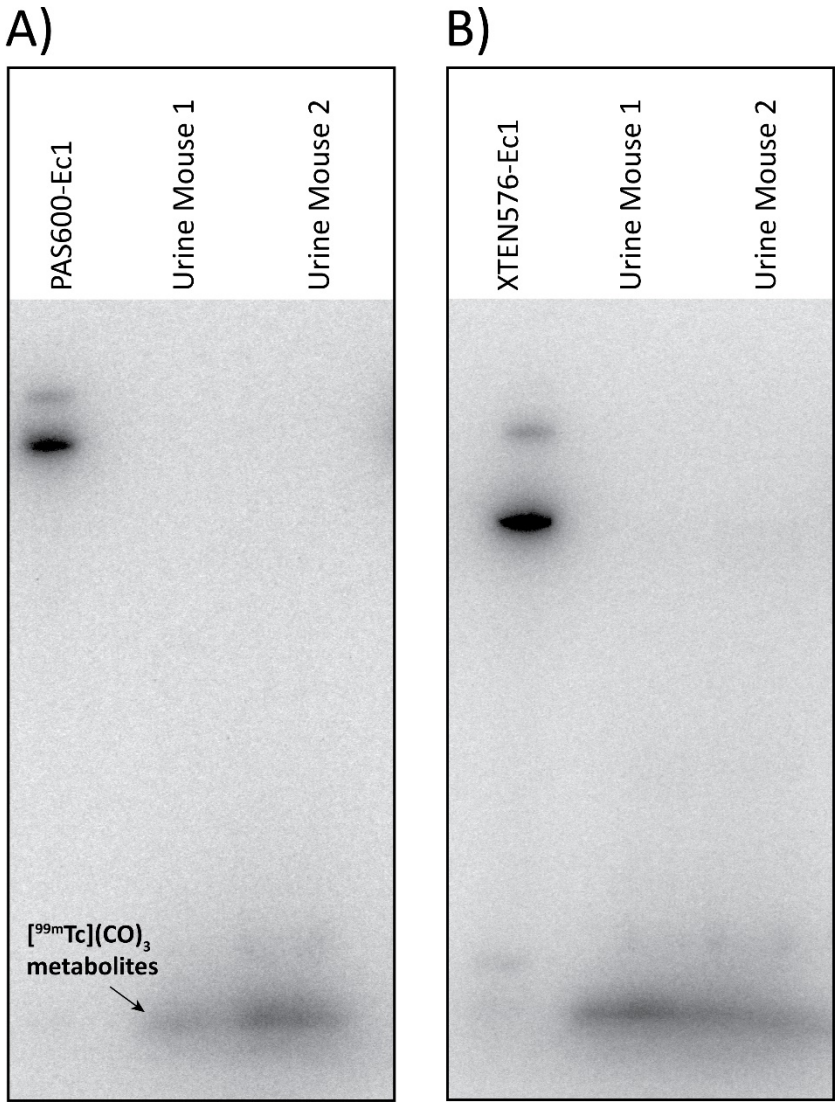


Figure S5: *Exemplary autoradiographs of urine samples separated on SDS-PAGE. Mice (N = 2) were i.v. injected with 532 pmol radiolabeled fusion protein with 7.5 MBq activity. Twenty-four hours after i.v. injection the mice were euthanized after SPECT/CT imaging and the urine collected post mortem. Radiolabeled reference samples (10 µL) which were i.v. injected into the mice and urine samples (20-50 µL) were separated on 4-12% NuPAGE Novex Bis-Tris SDS-PAGE gels. Gels were then placed on multisensitive phosphor imager plates (Perkin Elmer) for 16 h. Plates were imaged on a cyclone phosphor imager (Perkin Elmer). A) PAS600-Ec1 and B) XTEN576-Ec1*

Table S1: *Determination of absolute molecular weight and monodispersity of polypeptide-DARPin fusion proteins by size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS).*

Construct	$MW_{calc.}$ (kDa)	$MW_{abs.}$ (kDa)	ΔMW (%)	Mw/Mn
PAS300-Ec1	44.92	46.16	2.8	1.00
PAS600-Ec1	69.48	71.45	2.8	1.00
PAS900-Ec1	94.05	97.45	3.6	1.00
XTEN288-Ec1	46.93	47.74	1.7	1.00
XTEN576-Ec1	73.21	72.22	-1.3	1.00
XTEN864-Ec1	99.63	99.92	0.3	1.00

Calculated molecular weights (MW_{calc}) were obtained with the ProtParam tool on the ExPASy calculation server, based on the amino acid sequence. The absolute molecular weight (MW_{abs}) was determined by SEC-MALS and the deviation of the MW_{abs} of the MW_{calc} corresponds MW_{abs}/MW_{calc} in percent. The polydispersity index Mw/Mn = 1.00 indicates that the analyzed protein samples were monodisperse.

Table S2: *Biodistribution of polypeptide-DARPin fusion proteins and unmodified DARPins Ec1 and Off7 in mice bearing EpCAM-positive HT29 xenografts. Mice (N = 3 per time point) were euthanized 1, 4, 24 and 48 h after i.v. injection of 413 pmol $^{99m}Tc(CO_3)_2$ radiolabeled protein with 1 MBq specific activity. Activity in organs was measured with a γ-scintillation counter. Data are given as percentage of injected dose per gram of tissue (%ID/g) and shown as average ± SD.*

	PAS300-Ec1				**PAS300-Off7**			
%ID/g	**1 h**	**4 h**	**24 h**	**48 h**	**1 h**	**4 h**	**24 h**	**48 h**
Tumor	2.21 ± 0.66	5.91 ± 0.90	6.83 ± 1.09	9.11 ± 0.58	1.68 ± 0.36	2.87 ± 0.51	2.41 ± 0.26	1.33 ± 0.18
Blood	25.74 ± 4.85	14.28 ± 0.83	2.03 ± 0.10	0.79 ± 0.12	16.38 ± 2.06	7.25 ± 1.58	0.40 ± 0.10	0.13 ± 0.01
Heart	9.04 ± 2.57	7.21 ± 1.55	1.77 ± 0.01	1.07 ± 0.21	6.14 ± 0.55	3.69 ± 1.49	1.11 ± 0.13	0.75 ± 0.17
Lung	10.52 ± 2.16	6.51 ± 0.77	1.92 ± 0.68	0.98 ± 0.21	6.46 ± 1.70	3.80 ± 0.64	0.85 ± 0.16	0.48 ± 0.06
Stomach	5.63 ± 1.09	4.85 ± 0.19	1.89 ± 0.65	1.61 ± 0.16	4.39 ± 0.17	4.83 ± 0.27	1.69 ± 0.56	1.62 ± 0.25
Intestine	3.92 ± 0.77	3.23 ± 0.51	1.45 ± 0.28	1.17 ± 0.36	3.40 ± 0.72	2.68 ± 0.45	1.28 ± 0.58	0.85 ± 0.22
Bone	2.10 ± 0.18	2.41 ± 0.33	1.23 ± 0.15	0.62 ± 0.37	2.55 ± 1.12	3.35 ± 1.09	2.57 ± 0.97	1.33 ± 0.33
Muscle	0.70 ± 0.03	0.92 ± 0.07	0.51 ± 0.08	0.32 ± 0.11	0.55 ± 0.08	0.61 ± 0.04	0.47 ± 0.10	0.27 ± 0.03
Spleen	5.30 ± 1.57	5.23 ± 0.62	4.06 ± 0.82	4.32 ± 1.08	10.47 ± 2.94	11.04 ± 3.49	6.57 ± 1.70	3.45 ± 0.52
Kidney	24.98 ± 4.58	47.83 ± 4.86	45.85 ± 5.10	33.75 ± 2.01	31.62 ± 9.80	50.60 ± 4.53	37.17 ± 4.76	17.41 ± 3.37
Liver	8.01 ± 1.26	9.32 ± 0.66	8.50 ± 1.18	8.16 ± 1.03	21.98 ± 4.01	26.27 ± 2.73	15.79 ± 2.43	8.58 ± 0.68
Pancreas	4.50 ± 1.17	4.42 ± 0.64	2.40 ± 0.64	2.06 ± 0.22	4.46 ± 0.56	3.58 ± 0.26	2.32 ± 0.43	1.45 ± 0.16
Skin	2.22 ± 0.42	3.72 ± 1.08	2.99 ± 0.30	3.03 ± 0.93	1.89 ± 0.45	3.24 ± 0.48	2.72 ± 0.33	1.78 ± 0.08
	PAS600-Ec1				**PAS600-Off7**			
%ID/g	1 h	4 h	24 h	48 h	1 h	4 h	24 h	48 h
Tumor	1.89 ± 0.04	5.39 ± 0.28	9.68 ± 0.55	9.13 ± 1.49	2.55 ± 0.59	3.89 ± 0.93	3.94 ± 0.03	3.35 ± 0.10
Blood	26.26 ± 1.15	23.48 ± 1.24	7.04 ± 0.12	2.68 ± 0.26	26.77 ± 0.94	19.17 ± 1.52	3.21 ± 0.39	0.83 ± 0.08
Heart	10.97 ± 1.05	7.86 ± 1.72	2.99 ± 0.20	1.76 ± 0.28	9.92 ± 1.11	5.88 ± 1.92	1.74 ± 0.39	1.23 ± 0.08
Lung	12.01 ± 0.54	10.57 ± 1.04	3.28 ± 0.12	2.32 ± 0.48	13.62 ± 1.13	8.21 ± 0.50	1.93 ± 0.35	1.11 ± 0.16
Stomach	4.35 ± 0.19	5.61 ± 0.23	3.06 ± 0.09	2.18 ± 0.21	4.93 ± 0.62	5.54 ± 0.97	3.20 ± 0.32	3.17 ± 0.09
Intestine	3.42 ± 0.27	3.27 ± 0.42	1.68 ± 0.31	1.10 ± 0.06	3.44 ± 0.49	3.02 ± 0.18	1.42 ± 0.24	1.59 ± 0.29
Bone	2.33 ± 0.32	2.22 ± 0.13	1.41 ± 0.18	1.76 ± 0.87	3.12 ± 0.22	2.61 ± 1.03	1.45 ± 0.08	1.26 ± 0.10
Muscle	0.58 ± 0.12	0.89 ± 0.19	0.60 ± 0.03	0.38 ± 0.09	0.59 ± 0.13	0.82 ± 0.21	0.55 ± 0.14	0.48 ± 0.13
Spleen	4.77 ± 0.45	4.88 ± 0.18	5.02 ± 0.20	4.14 ± 1.04	6.33 ± 0.70	5.96 ± 0.55	4.64 ± 0.75	5.24 ± 0.54
Kidney	12.60 ± 0.76	16.08 ± 1.51	21.95 ± 3.82	17.50 ± 3.38	14.59 ± 0.33	21.48 ± 3.02	18.49 ± 2.32	16.33 ± 3.75
Liver	7.90 ± 0.55	8.69 ± 0.66	7.64 ± 0.46	7.11 ± 0.87	12.09 ± 0.55	13.59 ± 1.80	11.38 ± 1.69	10.72 ± 0.99
Pancreas	3.92 ± 0.6	4.84 ± 0.47	3.31 ± 0.33	2.86 ± 0.18	4.31 ± 0.19	5.42 ± 1.07	3.56 ± 0.45	3.32 ± 0.35
Skin	1.65 ± 0.46	3.56 ± 0.85	3.96 ± 0.25	4.33 ± 1.59	1.82 ± 0.61	11.34 ± 11.15	4.88 ± 0.78	4.77 ± 1.01
	PAS900-Ec1				**PAS900-Off7**			
%ID/g	1 h	4 h	24 h	48 h	1 h	4 h	24 h	48 h
Tumor	1.52 ± 0.26	3.47 ± 0.55	10.48 ± 3.33	10.79 ± 1.69	2.29 ± 1.10	2.94 ± 0.63	3.79 ± 0.14	3.79 ± 1.27
Blood	34.20 ± 4.24	30.04 ± 3.93	12.71 ± 1.92	5.73 ± 1.86	36.49 ± 2.21	23.51 ± 1.40	10.68 ± 2.01	3.73 ± 0.58
Heart	13.00 ± 4.28	10.19 ± 1.15	5.33 ± 1.06	3.41 ± 0.93	18.44 ± 8.89	9.42 ± 1.45	5.44 ± 2.81	1.88 ± 0.18
Lung	14.41 ± 3.73	11.47 ± 0.90	5.26 ± 0.72	4.08 ± 1.47	16.51 ± 4.10	9.42 ± 0.74	4.44 ± 1.03	2.00 ± 0.17
Stomach	2.71 ± 0.79	4.07 ± 0.34	3.03 ± 0.31	2.98 ± 0.33	4.03 ± 0.27	4.16 ± 0.40	3.25 ± 0.54	2.54 ± 0.28
Intestine	1.85 ± 0.20	2.38 ± 0.11	1.45 ± 0.05	2.35 ± 0.59	2.79 ± 0.59	1.89 ± 0.27	1.45 ± 0.37	1.15 ± 0.32
Bone	2.45 ± 0.75	3.34 ± 1.50	1.68 ± 0.21	2.53 ± 1.87	3.92 ± 0.95	2.55 ± 0.37	1.67 ± 0.42	1.78 ± 0.45
Muscle	0.53 ± 0.19	0.60 ± 0.01	0.70 ± 0.18	0.94 ± 0.61	0.78 ± 0.25	0.62 ± 0.14	0.73 ± 0.25	0.42 ± 0.07
Spleen	4.63 ± 0.88	6.07 ± 0.55	6.24 ± 1.25	7.10 ± 2.30	16.06 ± 2.38	10.27 ± 0.23	7.72 ± 1.86	4.44 ± 0.57
Kidney	8.17 ± 0.67	9.00 ± 1.94	10.29 ± 1.83	9.70 ± 0.46	11.37 ± 2.10	9.56 ± 1.34	10.28 ± 2.68	8.20 ± 0.91
Liver	7.36 ± 1.64	7.53 ± 0.83	7.31 ± 0.82	7.62 ± 0.48	14.78 ± 1.23	11.37 ± 0.42	11.45 ± 2.45	9.36 ± 0.40
Pancreas	2.78 ± 0.07	3.20 ± 0.28	2.52 ± 0.24	3.69 ± 1.06	3.70 ± 0.17	3.63 ± 0.77	2.93 ± 0.56	2.36 ± 0.13
Skin	1.63 ± 0.38	2.87 ± 1.30	5.83 ± 0.66	5.86 ± 1.12	2.17 ± 0.58	4.09 ± 1.44	4.76 ± 0.83	4.77 ± 0.29

	XTEN288-Ec1				XTEN288-Off7			
%ID/g	1 h	4 h	24 h	48 h	1 h	4 h	24 h	48 h
Tumor	1.51 ± 0.34	5.03 ± 1.16	7.79 ± 2.18	4.98 ± 0.92	1.37 ± 0.30	1.88 ± 0.31	1.00 ± 0.12	1.17 ± 0.34
Blood	16.99 ± 0.36	14.82 ± 0.22	3.03 ± 1.05	0.79 ± 0.18	14.11 ± 1.05	4.55 ± 0.79	0.31 ± 0.01	0.15 ± 0.01
Heart	8.22 ± 2.00	6.20 ± 1.77	1.72 ± 0.43	0.73 ± 0.13	5.36 ± 0.74	2.09 ± 0.61	0.62 ± 0.06	0.53 ± 0.10
Lung	5.98 ± 0.72	6.04 ± 0.66	1.88 ± 0.76	0.92 ± 0.28	5.71 ± 0.77	2.37 ± 0.72	0.47 ± 0.02	0.41 ± 0.11
Stomach	3.75 ± 0.15	3.23 ± 2.06	1.98 ± 0.52	1.45 ± 0.21	4.32 ± 0.40	3.51 ± 0.68	1.55 ± 0.47	1.12 ± 0.40
Intestine	2.64 ± 0.13	2.67 ± 0.41	1.11 ± 0.23	0.77 ± 0.17	3.03 ± 0.40	1.70 ± 0.30	0.87 ± 0.15	0.60 ± 0.22
Bone	1.71 ± 0.26	2.59 ± 0.33	1.34 ± 0.30	0.62 ± 0.03	2.23 ± 0.73	1.55 ± 0.04	0.84 ± 0.21	1.06 ± 0.63
Muscle	0.56 ± 0.12	0.86 ± 0.22	0.58 ± 0.23	0.28 ± 0.10	0.53 ± 0.05	0.44 ± 0.13	0.30 ± 0.01	0.27 ± 0.14
Spleen	3.02 ± 0.16	6.63 ± 2.51	4.82 ± 1.46	3.57 ± 0.97	4.11 ± 0.25	3.27 ± 0.52	2.20 ± 0.14	1.63 ± 0.21
Kidney	40.47 ± 4.70	105.68 ± 10.49	114.46 ± 22.43	51.90 ± 9.26	59.31 ± 3.24	82.48 ± 19.04	60.30 ± 4.01	31.45 ± 7.65
Liver	5.34 ± 0.33	8.78 ± 0.35	8.97 ± 1.65	5.07 ± 0.41	10.31 ± 1.14	10.14 ± 2.89	6.15 ± 1.13	4.62 ± 0.44
Pancreas	4.32 ± 0.16	4.22 ± 0.31	2.08 ± 0.45	1.18 ± 0.41	4.32 ± 0.40	2.58 ± 0.07	1.66 ± 0.26	1.41 ± 0.25
Skin	1.77 ± 0.23	3.20 ± 0.81	3.22 ± 0.88	2.13 ± 0.43	2.16 ± 0.18	2.36 ± 0.69	1.70 ± 0.05	1.55 ± 0.15

	XTEN576-Ec1				XTEN576-Off7			
%ID/g	1 h	4 h	24 h	48 h	1 h	4 h	24 h	48 h
Tumor	1.82 ± 0.31	2.99 ± 1.15	6.44 ± 1.40	6.88 ± 2.13	1.69 ± 0.32	3.75 ± 1.61	3.81 ± 0.15	3.38 ± 0.26
Blood	29.79 ± 1.74	22.41 ± 2.33	7.38 ± 0.78	2.41 ± 0.10	26.50 ± 0.87	18.88 ± 0.78	4.12 ± 0.99	1.01 ± 0.13
Heart	9.69 ± 1.35	6.90 ± 1.35	2.81 ± 0.17	1.31 ± 0.10	9.16 ± 2.18	5.67 ± 0.42	1.70 ± 0.33	0.97 ± 0.17
Lung	12.72 ± 2.58	9.33 ± 0.82	3.10 ± 0.66	1.41 ± 0.16	11.80 ± 1.60	8.60 ± 0.55	1.90 ± 0.48	0.84 ± 0.08
Stomach	5.16 ± 0.46	4.80 ± 0.68	2.90 ± 0.29	2.04 ± 0.11	4.74 ± 0.79	5.37 ± 0.20	3.85 ± 0.48	3.29 ± 0.80
Intestine	2.62 ± 0.18	2.30 ± 0.28	1.30 ± 0.11	0.85 ± 0.23	2.88 ± 0.22	2.45 ± 0.25	1.19 ± 0.27	0.84 ± 0.06
Bone	2.70 ± 0.35	1.99 ± 0.40	1.27 ± 0.20	0.80 ± 0.26	2.27 ± 0.17	2.26 ± 0.58	1.39 ± 0.14	0.97 ± 0.39
Muscle	0.54 ± 0.12	0.48 ± 0.11	0.55 ± 0.03	0.45 ± 0.03	1.11 ± 0.63	0.63 ± 0.14	0.50 ± 0.03	0.41 ± 0.03
Spleen	4.72 ± 0.56	4.26 ± 0.96	3.84 ± 0.33	4.02 ± 0.55	5.64 ± 0.52	5.31 ± 0.85	4.10 ± 1.14	3.79 ± 0.43
Kidney	12.89 ± 0.63	16.17 ± 3.43	25.26 ± 2.86	21.54 ± 1.68	13.81 ± 2.36	22.27 ± 0.18	26.01 ± 2.05	15.51 ± 0.57
Liver	9.02 ± 0.21	7.87 ± 1.30	6.03 ± 0.40	6.07 ± 0.33	10.90 ± 0.55	10.66 ± 1.14	10.22 ± 0.44	7.55 ± 0.85
Pancreas	4.18 ± 0.39	4.21 ± 0.41	3.16 ± 0.15	3.19 ± 0.10	4.64 ± 0.27	5.02 ± 0.09	3.72 ± 0.13	3.08 ± 0.19
Skin	2.13 ± 0.71	3.16 ± 0.59	4.24 ± 0.74	3.68 ± 0.22	1.66 ± 0.32	3.58 ± 0.22	4.72 ± 0.47	3.92 ± 0.32

	XTEN864-Ec1				XTEN864-Off7			
%ID/g	1 h	4 h	24 h	48 h	1 h	4 h	24 h	48 h
Tumor	1.13 ± 0.18	3.03 ± 0.58	10.62 ± 2.42	9.90 ± 1.46	1.36 ± 0.05	2.50 ± 0.47	5.01 ± 0.97	4.04 ± 2.18
Blood	34.32 ± 2.15	29.53 ± 2.16	14.93 ± 1.60	6.70 ± 0.45	25.11 ± 1.56	23.66 ± 0.89	10.95 ± 0.53	4.13 ± 0.27
Heart	12.07 ± 4.10	9.66 ± 0.26	5.83 ± 1.84	2.96 ± 0.42	9.99 ± 1.58	6.50 ± 0.79	2.96 ± 0.68	1.67 ± 0.31
Lung	12.98 ± 1.01	10.68 ± 1.98	5.85 ± 0.70	3.03 ± 0.50	11.21 ± 2.25	10.41 ± 1.13	4.24 ± 0.33	2.03 ± 0.48
Stomach	2.57 ± 0.10	3.63 ± 0.06	2.97 ± 0.08	2.40 ± 0.17	3.24 ± 0.61	4.35 ± 0.69	3.18 ± 0.39	2.59 ± 0.42
Intestine	1.74 ± 0.15	2.01 ± 0.13	1.40 ± 0.15	1.33 ± 0.22	2.20 ± 0.31	2.05 ± 0.23	1.19 ± 0.06	0.97 ± 0.10
Bone	2.38 ± 0.96	2.34 ± 0.36	1.66 ± 0.21	1.61 ± 0.54	2.34 ± 0.25	2.26 ± 0.37	1.55 ± 0.15	0.83 ± 0.16
Muscle	0.47 ± 0.08	0.48 ± 0.07	0.72 ± 0.06	0.74 ± 0.23	0.53 ± 0.08	0.55 ± 0.07	0.44 ± 0.25	0.41 ± 0.12
Spleen	4.15 ± 0.51	4.99 ± 1.05	5.71 ± 0.89	5.79 ± 0.69	5.29 ± 0.45	7.02 ± 1.88	6.17 ± 1.01	5.51 ± 0.76
Kidney	7.48 ± 0.16	8.28 ± 1.25	9.72 ± 1.21	8.28 ± 1.23	8.03 ± 1.31	8.58 ± 0.59	7.51 ± 0.91	6.50 ± 0.69
Liver	6.37 ± 0.59	6.79 ± 1.06	7.01 ± 0.58	6.45 ± 0.40	9.65 ± 0.84	10.50 ± 0.40	9.93 ± 1.48	8.12 ± 0.28
Pancreas	2.11 ± 0.12	3.25 ± 0.38	3.08 ± 0.26	2.59 ± 0.64	2.34 ± 0.42	3.36 ± 0.40	3.47 ± 0.54	3.01 ± 0.49
Skin	1.76 ± 0.27	2.98 ± 0.84	4.98 ± 1.44	6.23 ± 1.17	2.26 ± 0.31	3.68 ± 0.73	4.75 ± 1.38	4.91 ± 1.54

	Ec1				Off7			
%ID/g	1 h	4 h	24 h	48 h	1 h	4 h	24 h	48 h
Tumor	1.46 ± 0.14	2.46 ± 0.39	2.05 ± 0.36	1.66 ± 0.09	1.13 ± 0.10	1.11 ± 0.22	0.56 ± 0.13	0.32 ± 0.16
Blood	0.68 ± 0.02	0.46 ± 0.03	0.21 ± 0.04	0.12 ± 0.01	0.54 ± 0.05	0.29 ± 0.04	0.13 ± 0.01	0.07 ± 0.02
Heart	0.90 ± 0.08	0.82 ± 0.12	0.57 ± 0.05	0.41 ± 0.17	1.32 ± 0.21	0.89 ± 0.19	0.53 ± 0.03	0.30 ± 0.06
Lung	0.94 ± 0.13	0.86 ± 0.06	0.72 ± 0.27	0.35 ± 0.05	2.55 ± 0.37	1.79 ± 0.29	0.95 ± 0.28	0.42 ± 0.04
Stomach	1.29 ± 0.18	1.72 ± 0.08	0.88 ± 0.37	0.66 ± 0.06	1.53 ± 0.27	1.33 ± 0.06	0.60 ± 0.19	0.53 ± 0.10
Intestine	1.11 ± 0.16	1.19 ± 0.20	0.78 ± 0.27	0.60 ± 0.10	1.20 ± 0.11	1.02 ± 0.07	0.57 ± 0.11	0.34 ± 0.07
Bone	1.44 ± 0.22	2.26 ± 0.17	1.67 ± 0.31	1.67 ± 1.22	3.89 ± 1.44	2.98 ± 0.22	1.97 ± 0.28	0.93 ± 0.33
Muscle	0.33 ± 0.06	0.34 ± 0.04	0.25 ± 0.04	0.18 ± 0.08	0.60 ± 0.22	0.41 ± 0.09	0.24 ± 0.07	0.14 ± 0.05
Spleen	2.41 ± 0.19	3.38 ± 0.76	2.77 ± 0.72	2.01 ± 0.67	8.32 ± 2.05	6.11 ± 0.85	3.62 ± 1.12	2.23 ± 0.86
Kidney	82.20 ± 4.06	111.35 ± 20.48	86.53 ± 17.71	56.67 ± 11.97	129.46 ± 21.98	100.98 ± 7.20	63.96 ± 6.47	33.90 ± 7.99
Liver	13.82 ± 0.55	18.56 ± 1.31	11.20 ± 2.89	7.21 ± 1.33	28.14 ± 3.81	24.06 ± 3.50	12.45 ± 1.28	6.94 ± 1.53
Pancreas	1.13 ± 0.20	1.12 ± 0.22	0.75 ± 0.21	0.66 ± 0.16	1.47 ± 0.05	1.08 ± 0.13	0.71 ± 0.04	0.40 ± 0.04
Skin	1.30 ± 0.22	1.39 ± 0.15	1.17 ± 0.34	0.72 ± 0.04	2.03 ± 0.09	1.49 ± 0.25	0.87 ± 0.01	0.52 ± 0.13

Table S3: *Detection of polypeptide-DARPin fusion proteins and unmodified DARPins Ec1 and Off7 in the feces and urine of mice bearing EpCAM-positive HT29 tumor xenografts.*

Construct	%ID Feces			%ID Urine		
Ec1	0.54	±	0.41	1.22	±	0.69
PAS300-Ec1	0.33	±	0.11	1.43	±	0.31
PAS600-Ec1	0.56	±	0.17	0.66	±	0.53
PAS900-Ec1	0.68	±	0.18	1.22	±	0.59
XTEN288-Ec1	0.24	±	0.17	1.33	±	0.19
XTEN576-Ec1	0.47	±	0.11	1.21	±	0.40
XTEN864-Ec1	0.37	±	0.14	0.44	±	0.08
Off7	0.45	±	0.11	0.93	±	0.66
PAS300-Off7	0.43	±	0.28	2.27	±	0.07
PAS600-Off7	0.51	±	0.20	0.85	±	0.35
PAS900-Off7	0.78	±	0.32	1.34	±	0.94
XTEN288-Off7	0.24	±	0.16	0.79	±	0.40
XTEN576-Off7	0.32	±	0.07	0.48	±	0.68
XTEN864-Off7	0.29	±	0.09	0.99	±	0.58

Mice bearing EpCAM positive HT29 tumor xenografts (N = 3 per time point) were euthanized 24 h after i.v. injection of 532 pmol [^{99m}Tc](CO)$_2$ radiolabeled protein with 7.5 MBq specific activity. Feces and urine were collected post mortem and activity was measured with a γ-scintillation counter. Data are given as percentage of injected dose of feces and urine and shown as average ± S.D.