

Supplementary information

Generation of a novel therapeutic peptide that depletes MDSC in tumor-bearing mice

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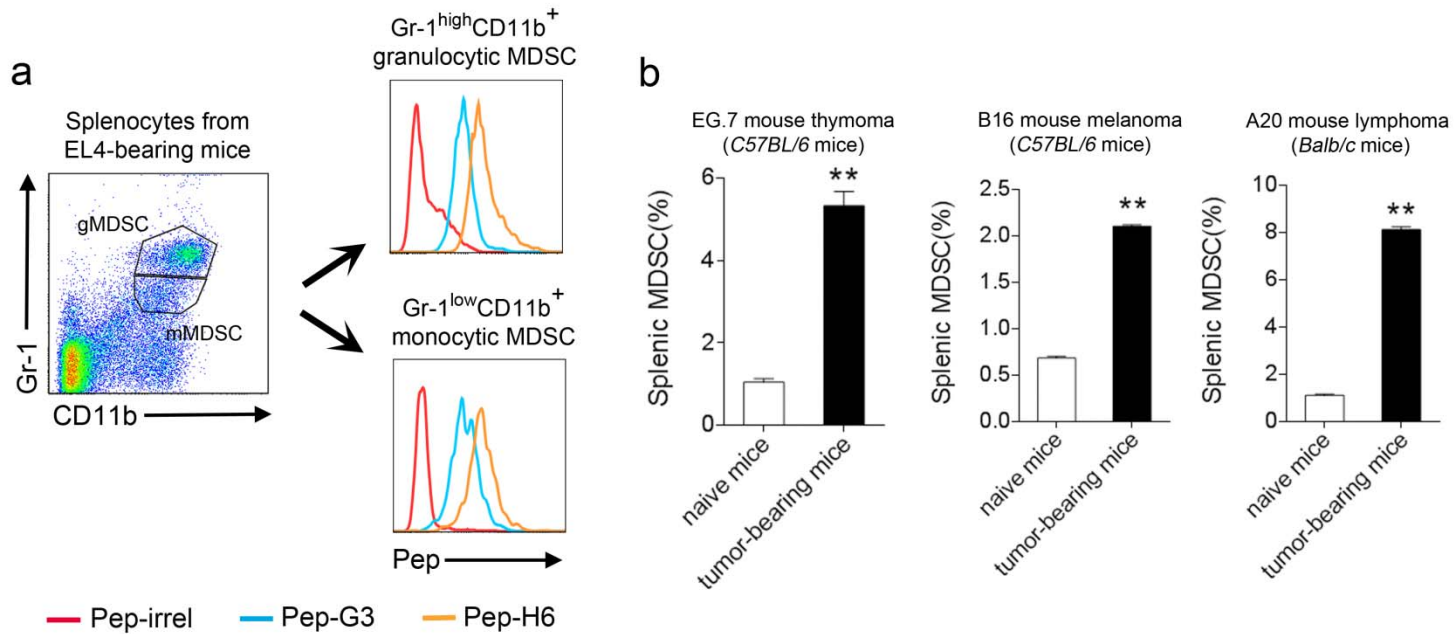
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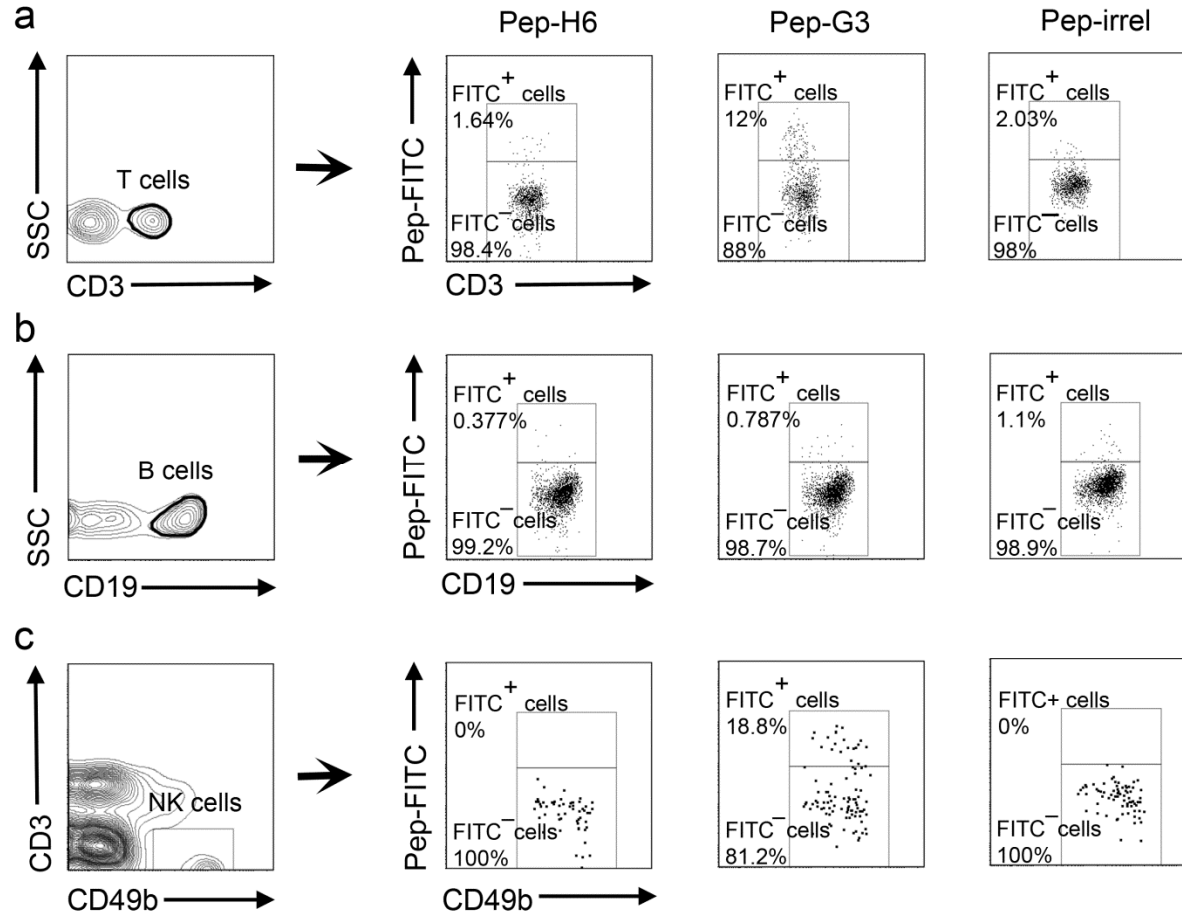
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Supplementary Figure 1



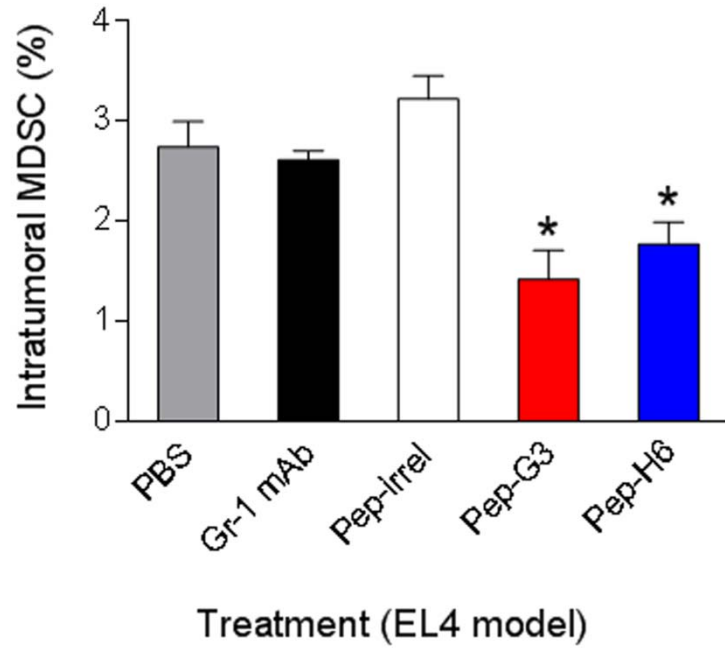
Supplementary Figure 1. Peptibodies recognized MDSC in tumor-bearing mice. (a) Binding of FITC-conjugated Pep-H6 or Pep-G3 on Gr-1^{high}CD11b⁺ gated granulocytic MDSC (gMDSC) and Gr-1^{low}CD11b⁺ gated monocytic MDSC (mMDSC) in splenocytes from EL4-bearing *C57BL/6* mice ($n = 5$). A non-specific peptibody (Pep-irrel) was used as a negative control. (b) Increase of MDSC in tumor-challenged mice. Splenocytes from naive mice or mice challenged with various tumors ($n = 3$ for each tumor type) were stained for CD11b and Gr-1 to identify MDSC. Frequencies of splenic MDSC are shown as mean \pm s.e.m. (** $P < 0.01$ compared with naïve mice by two-tailed student's t test).

Supplementary Figure 2



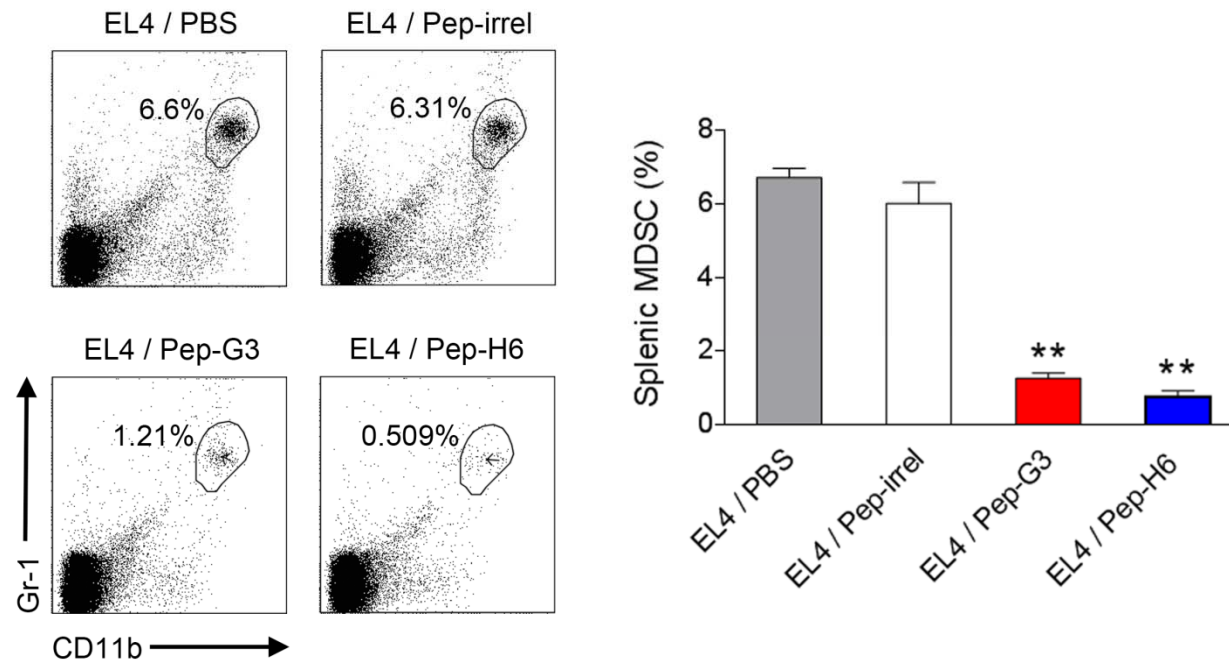
Supplementary Figure 2. Lack of peptibody binding on lymphocyte subsets. Splenocytes pooled from EL4-bearing *C57BL/c* mice ($n = 5$) were gated on $CD3^+$ T cells (a), $CD19^+$ B cells (b) and $CD3^-CD49b^+$ NK cells (c), respectively, and analyzed for peptibody binding.

Supplementary Figure 3



Supplementary Figure 3. Depletion of intratumoral MDSC in EL4 tumor-challenged mice by peptibody treatment. Groups of 5 *C57BL/6* mice were challenged s.c. with EL4 tumor cells followed by peptibody treatment as in Fig. 3a. The percentage of Gr-1⁺CD11b⁺ MDSC from single cell suspensions prepared from tumors harvested on Day 20 is shown as mean \pm s.e.m. (* $P < 0.01$ compared with PBS by two-tailed student's *t* test).

Supplementary Figure 4



Supplementary Figure 4. Long-term administration did not diminish peptibody-induced MDSC depletion. As in Fig. 3g, EL4-bearing *C57BL/6* mice were treated with peptibodies every other day for 2 weeks. At the end of treatment, splenocytes were harvested and stained for Gr-1⁺CD11b⁺ MDSC shown as the mean \pm s.e.m. of 5 mice per group. Plots are shown with frequency of splenic MDSC for individual representative mice. Differences between groups were analyzed by two-tailed Student's *t*-test. The data represent 2 independent experiments.

Supplementary Figure 5

[S10A9_MOUSE](#) Mass: 13211 Score: 127 Matches: 8(5) Sequences: 4(3) emPAI: 2.69
 Protein S100-A9 OS=Mus musculus GN=S100a9 PE=1 SV=3

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
24	409.6955	817.3764	817.3752	1.51	0	27	0.051	1	U	K.APSQMER.S
729	587.2882	1172.5619	1172.5608	0.94	1	(25)	0.1	1	U	M_ANKAPSQMER.S 728
760	595.2852	1188.5558	1188.5557	0.07	1	26	0.08	1	U	M_ANKAPSQMER.S
1201	690.3394	1378.6643	1378.6625	1.30	0	53	0.0002	1	U	R_QMVEAQLATFMK.K
1580	841.4318	1680.8491	1680.8471	1.16	0	54	0.00017	1	U	R.SITTIIDTFHQYSR.K 1581 1582

MANKAPSQMERSITTIIDTFHQYSRKEGHPDTLSKKEFRQMVEAQLATFMKKEKRNEAL
 INDIMEDLDTNQDNQLSFEECMMLMAKLI FACHEKHLHENNPRGHGHS HGKGC GK

Protein sequence coverage: 35.4%

[S10A8_MOUSE](#) Mass: 10345 Score: 552 Matches: 12(12) Sequences: 2(2) emPAI: 2.72
 Protein S100-A8 OS=Mus musculus GN=S100a8 PE=1 SV=3

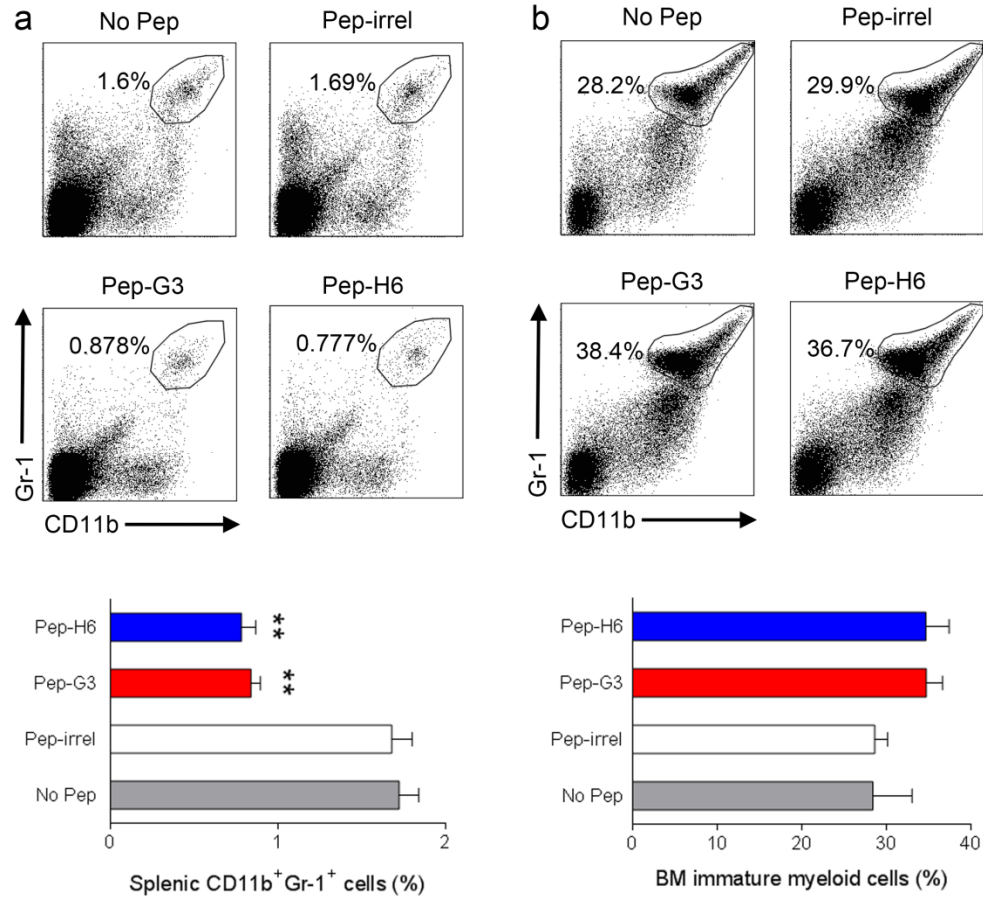
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2756	809.0721	2424.1946	2424.1882	2.62	0	(55)	0.00014	1	U	R.ELDINSDNAINFEEFLAMVIK.V 2751 2754
2783	1221.1024	2440.1903	2440.1832	2.92	0	(104)	1.5e-009	1	U	R.ELDINSDNAINFEEFLAMVIK.V 2782
3199	928.8013	2783.3822	2783.3779	1.53	0	68	5.5e-006	1	U	K.ALSNLIDVYHNYSNIQGNHHALYK.N 3190 3198 3200

MPSELEKALSNLIDVYHNYSNIQGNHHALYKNDFKKMVTTECPQFVQNININLENLFR
 ELDINSDNAINFEEFLAMVIKVGVASHKDSHKE

Protein sequence coverage: 55.1%

Supplementary Figure 5. Pep-G3 also immunoprecipitated S100 family proteins. Proteomic analysis of eluates of Pep-G3-bound, sorted MDSC lysates isolated from immobilized Protein A revealed predominant peptides with homology to S100A9 and A8 proteins. Control eluates of non-peptibody bound, sorted MDSC lysates from Protein A displayed peptides possessing homology only to keratin (not shown).

Supplementary Figure 6



Supplementary Figure 6. Peptibodies did not cause bone marrow toxicity. Frequencies of Gr1⁺CD11b⁺ splenic MDSC (a) or bone marrow immature myeloid cells (b) from naive C57BL/6 mice treated with peptibodies for 3 consecutive days. Untreated (No Pep) or Pep-irrel-treated mice served as negative controls. Data are shown as the mean ± s.e.m of 3 mice per group. ** *P* < 0.01 compared with untreated mice (No pep)] by two-tailed Student's *t*-test.

Supplementary Table 1. Peptibody treatment corrected aberrant neutrophilia in EL4-bearing C57BL/6 mice

	Non-tumor bearing			EL4 / PBS			EL4 / Pep-irrel			EL4 / Pep-G3			EL4 / Pep-H6		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
WBC ($10^3 \mu\text{l}^{-1}$)	2.9	8.68	3.72	18.4	14.79	19.86	12.82	19.26	19.92	5.12	3.3	4.05	2.84	3.28	2.78
RBC ($10^6 \mu\text{l}^{-1}$)	9.35	10.77	10.47	7.98	9.17	8.65	9.55	8.49	8.28	8	9.07	8.77	8.74	9.79	8.89
HGB (g dl ⁻¹)	13.8	16	15.8	11.6	13.2	12.6	13.5	12.1	12.2	11.5	13	12.5	12.8	14.4	13.4
PLT ($10^3 \mu\text{l}^{-1}$)	686	1167	975	721	1044	596	896	1125	721	559	506	750	694	1199	933
NEUT (%)	9.9	7.4	8.5	50	51.6	49	44	59.4	58	26	30	18	12	12.3	11
LYMPH (%)	85.3	88.2	85.7	35	40.6	40	45	31.7	33	59	63	79	84	81.9	82
MONO (%)	1.6	1.4	2.2	3	1.3	1	3	1.1	1	0	0	0	0	0.5	1
EOS (%)	2.7	1.3	1.5	7	3.9	3	5	4.7	5	2	0	1	2	2.1	0
BASO (%)	0.1	0.6	0.5	0	0.7	0	0	0.6	0	0	0	0	0	1.6	0

Reference values for mice blood test
(provided by Veterinary Laboratory Medicin in MD Anderosn Cancer Centger)

Hematology	Range	Female
WBC	$10^3 \mu\text{l}^{-1}$	2.1 – 7.1
RBC	$10^6 \mu\text{l}^{-1}$	7.4 – 9.9
HGB	g dl ⁻¹	12.1 – 16.5
Platelets	$10^3 \mu\text{l}^{-1}$	659 – 1427
Neutrophils	%	7.4 – 25.9
Lymphocytes	%	60.8 – 85.0
Monocytes	%	0.2 – 4.4
Eosinophils	%	0.0 – 13.0
Basophils	%	0.0 – 0.7

Supplementary Table 2. List of antibodies used in studies

Antibody	Clone number	Working concentration	Supplier	Catalog number
Anti-CD3-PerCP	145-2C11	1 $\mu\text{g ml}^{-1}$	BD Biosciences	553067
Anti-CD11b-APC	M1/70	1 $\mu\text{g ml}^{-1}$	BD Biosciences	553312
Anti-CD11b-PerCP	M1/70	1 $\mu\text{g ml}^{-1}$	BD Biosciences	550993
Anti-CD11c-PerCP	HL3	1 $\mu\text{g ml}^{-1}$	BD Biosciences	560584
Anti-CD19-APC	1D3	1 $\mu\text{g ml}^{-1}$	BD Biosciences	550992
Anti-CD49-PE	HM α 2	1 $\mu\text{g ml}^{-1}$	BD Biosciences	558759
Anti-Gr-1-FITC	RB6-8C5	1 $\mu\text{g ml}^{-1}$	BD Biosciences	553127
Anti-Gr-1-PE	RB6-8C5	1 $\mu\text{g ml}^{-1}$	BD Biosciences	553128
Anti-Ly6C-PerCP	HK1.4	1 $\mu\text{g ml}^{-1}$	BioLegend	128011
Anti-Ly6G-PE	1A8	1 $\mu\text{g ml}^{-1}$	BD Biosciences	551461
Goat anti-mouse IgG-HRP	pAb	1:10,000	Jackson ImmunoResearch	115-035-166
Anti 6 \times His-HRP	F24-796	1:1,000	BD Biosciences	552564
Anti-S100A9	2B10	1:1,000	Abcam	ab105472
Anti-S100A8	EPR3554	1:1,000	Abcam	ab92331
Goat anti-rat IgG-HRP	pAb	1:10,000	Jackson ImmunoResearch	112-035-167
Goat anti-rabbit IgG-HRP	pAb	1:10,000	Jackson ImmunoResearch	112-035-003