PNAS

Supporting Information for

Lipid nanoparticle-mediated lymph node-targeting delivery of cancer mRNA vaccine elicits robust CD8$^+$ T cell response

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Materials

All the chemicals for lipid synthesis were purchased from Sigma-Aldrich or Fisher Scientific. 96 well high binding plates for ELISA assay were purchased from Fisher Scientific (Pittsburgh, PA, USA). HRP-conjugated total IgG, IgG1, and IgG2c goat anti-mouse antibody were bought from Abcam (Boston, MA, USA). pMRNA plasmid for $TRP2_{180\text{-}188}$ mRNA construction was purchased from System Biosciences (CA, USA). B16F10 murine melanoma cells were purchased from ATCC (Manassas, VA, USA) and cultured in Dulbecco's modified eagle's medium (DMEM, Sigma-Aldrich) with 10% fetal bovine serum (FBS, Sigma-Aldrich) and 1% penicillin-streptomycin (Gibco). H-$2K^b$ ($OVA_{257\text{-}264}$)-SIINFEKL-PE tetramer was purchased from MBL International Corporation (Woburn, MA, USA). The labels and catalogs of antibodies are listed in Table S1. Luciferase and Cre mRNA were purchased from Trilink BioTechnologies (San Diego, CA, USA)

Chemical synthesis

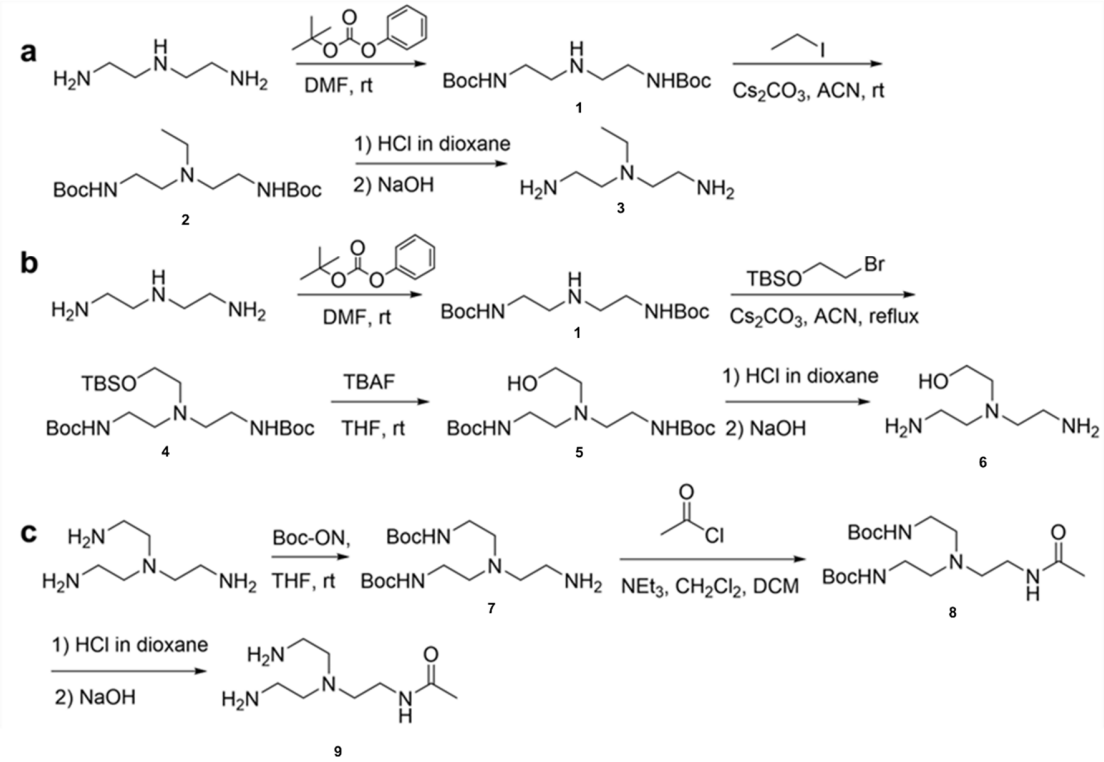

Fig. S1. The synthesis route of 113 analog head 3, 6 and 9

Synthesis of Amine 3. 1 was synthesized according to the known procedure.[1] A solution of 1 (1.21 g, 4 mmol) in 20 ml of acetonitrile, cesium carbonate (3.91 g, 12 mmol) and ethyl Iodide (0.39 ml, 4.8 mmol) was added successively under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature (r.t.). The reaction mixture was then concentrated under reduced pressure. Water (20 ml) and ethyl acetate (20 ml) was then added to the residue. The aqueous

layer was extracted twice with ethyl acetate (2*20 ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated with rotary evaporation. The residue was purified by silica gel column chromatography (Dichloromethane/Methanol = 95/5 to 90/10) to yield 1.06 g of 2 (yield 80%). ESI-MS m/z: $[M+H]^+$ calcd. 332.25; found 332.30.

A solution of 2 (995mg, 3 mmol) in 10 ml of ethyl acetate, was added dropwise 4N HCl in dioxane (4.5 ml, 18 mmol) at 0 ° C under nitrogen atmosphere. The reaction mixture was stirred at r.t.. Then the reaction mixture was concentrated under reduced pressure. The residue was triturated with diethyl ether three times and a small amount of ethyl acetate. The resultant solids were dissolved in 10N NaOH and extracted with dichloromethane (6* 20 ml). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to 280 mg of 3 (yield 71%). ESI-MS m/z: $[M+H]^+$ calcd. 132.14; found 132.26.

Synthesis of amine 6. 4 was prepared in a similar way as 2. A solution of 1 (1.21 g, 4 mmol) in 20 ml of acetonitrile, cesium carbonate (3.91 g, 12 mmol) and (2-bromoethoxy) (*tert*-butyl) Dimethyl silane (1.44 g, 6 mmol) was added successively under nitrogen atmosphere. The reaction mixture was stirred under reflux for one day. After the reaction was complete (monitored by TLC), the reaction mixture was cooled and concentrated under reduced pressure. Water (20 ml) and ethyl acetate (20 ml) was then added to the residue. The aqueous layer was extracted with ethyl acetate twice (2*20ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated with rotary evaporation. The residue was purified by silica gel column chromatography (Dichloromethane/Methanol = 95/5 to 90/10) to yield 1.30 g of 2 (yield 70%). ESI-MS m/z: $[M+H]^+$ calcd. 462.33; found 462.35.

8 ml of TBAF solution (8 mmol, 1 M in THF) was added dropwise into a solution of 4 (1.15 g, 2.5 mmol) in 10 ml of dry THF at 0 ° C under nitrogen atmosphere. The reaction mixture was stirred at r.t. for 3-5 hours before it was quenched with 10 ml of PBS buffer (pH 7.0, 10X) when no more starting materials were present. The mixture was extracted with ethyl acetate (3*10 ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Dichloromethane/Methanol = 95/5 to 90/10) to yield 0.70 g of 5 (yield 80%). ESI-MS m/z: $[M+H]^+$ calcd. 348.24; found 348.30.

4N HCl in dioxane (2.6 ml, 10.4 mmol) was added dropwise into a solution of 5 (600mg, 1.73mmol) in 6 ml of ethyl acetate at 0 ° C under nitrogen atmosphere. The reaction mixture was stirred at r.t. until no more starting materials existed. Then the reaction mixture was concentrated under reduced pressure. The residue was triturated with diethyl ether three times and a small amount of ethyl acetate. The resultant solids were dissolved in 10N NaOH and extracted with dichloromethane (6* 20 ml). The combined organic layers were dried over anhydrous sodium

sulfate, filtered, and concentrated to yield 130 mg of 6 (yield 50%). ESI-MS m/z: $[M+H]^+$ calcd. 148.14; found 148.22.

Synthesis of amine 9. 7 was synthesized according to established procedures.[2] Triethylamine (1.2 ml, 8 mmol) was added to a solution of 7 (1.39 g, 4 mmol) in 10 ml of dry dichloromethane, followed by the dropwise addition of acetyl chloride (0.43 ml, 6 mmol) at 0°C under nitrogen atmosphere. The reaction mixture was stirred at r.t. overnight before it was quenched with 10 ml of saturated $NaHCO_3$ and diluted with dichloromethane. The organic layer was washed successively with NH_4Cl, brine, water, and dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Dichloromethane/Methanol = 95/5 to 90/10) to yield 1.24 g of 8 (yield 80%). ESI-MS m/z: $[M+H]^+$ calcd. 389.27; found 389.45.

4N HCl in dioxane (3.8 ml, 15.2 mmol) was added dropwise into a solution of 8 (970mg, 2.5 mmol) in 10 ml of ethyl acetate at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at r.t. until no starting materials were detectable. Then the reaction mixture was concentrated under reduced pressure. The residue was triturated with diethyl ether three times and a small amount of ethyl acetate. The resultant solids were dissolved in 3N NaOH and extracted with dichloromethane (6* 20 ml). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to yield 280 mg of 6 (yield 60%). ESI-MS m/z: $[M+H]^+$ calcd. 189.28; found 189.42.

Table S1. Primers and TRP2 template used for gene cloning

Primer	**Sequence**
A109_pMRNA_ EcoRI_F	GAAGAAATATAAGAGAATTC
A110_pMRNA_ BamHI_R	CCGCAGAAGGCAGCGGATCC
Template	Sequence[α]
A267_TRP2	GAAGAAATATAAGAGAATTCGCCACCATGAGCGTGTATGATTTTTTTGTGTGGCTGTAAGGATCCGCTGCCTTCTGCGG

α TRP2 coding sequence was underlined.

Table S2. Antibodies used for flow cytometry

Target	Label	Provider	Catalog
CD3e	APC	eBioscience™	50-112-9569
B220	Brilliant Violet 650	BD biosciences	BDB563893
NK-1.1	APC-Cy7	BD biosciences	BDB560618
CD11c	PE-Cyanine7	eBioscience	50-154-55
F4/80	PerCP-Cyanine5.5	eBioscience	50-112-9034
IFN-γ	APC	Biolegend	50-169-921
CD8a	PE-Cy5	BD biosciences	BDB561094
CD4	APC-Cy7	BD biosciences	BDB561830
CD3	FITC	eBioscience	50-112-9706
FoxP3	APC	R&D system	FPK8214A025
CD45	Brilliant Violet 510	BD biosciences	BDB563891
MHC Class II	PE	eBioscience	50-108-18
CD11b	APC/Cy7	Biolegend	50-162-532
CD163	PE/Cyanine7	Biolegend	155319
CD86	PE	BD biosciences	BDB561129

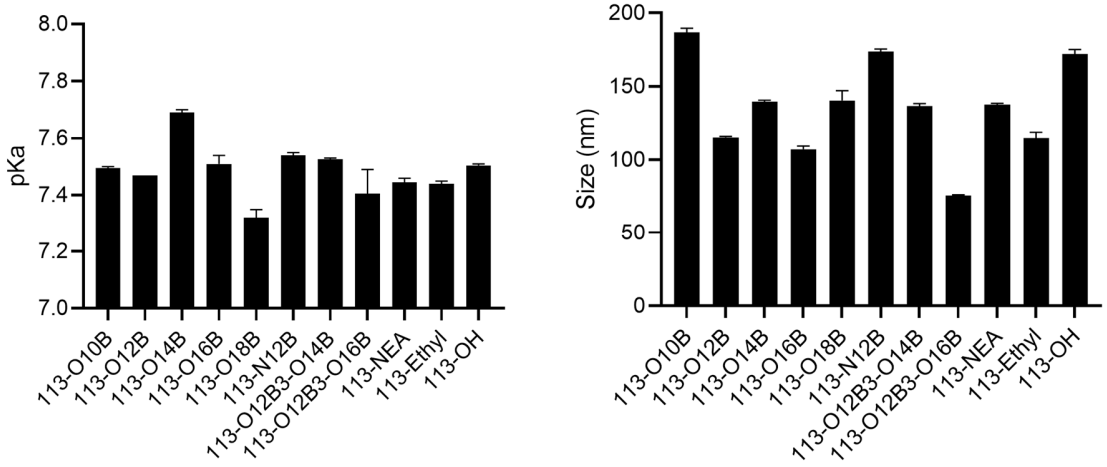

Fig. S2. pKa and size of different LNPs

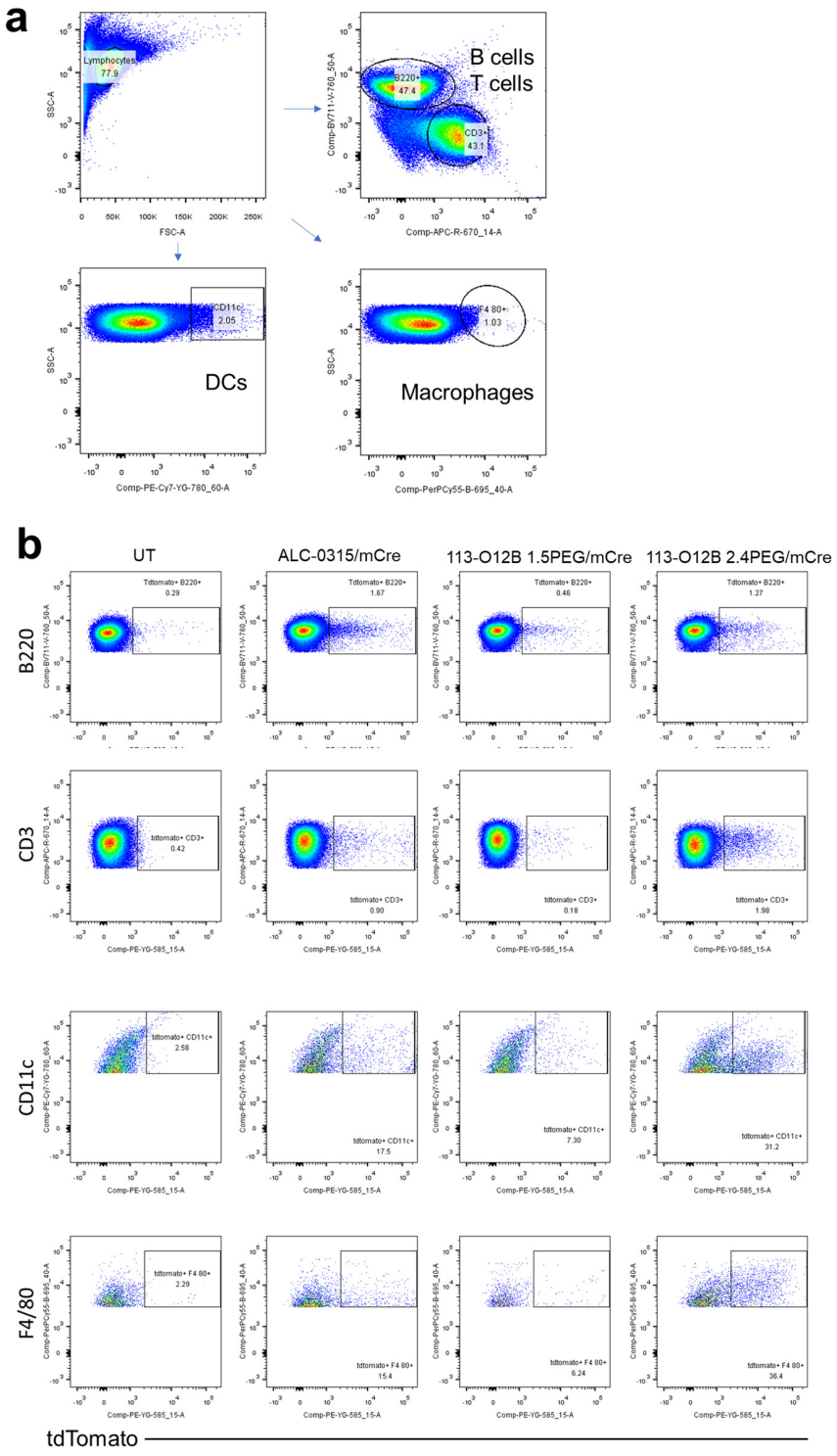

Fig. S3. Representative diagrams of flow cytometry for analysis of tdTomato positive cells with LNs after treatment with LNP/mCre

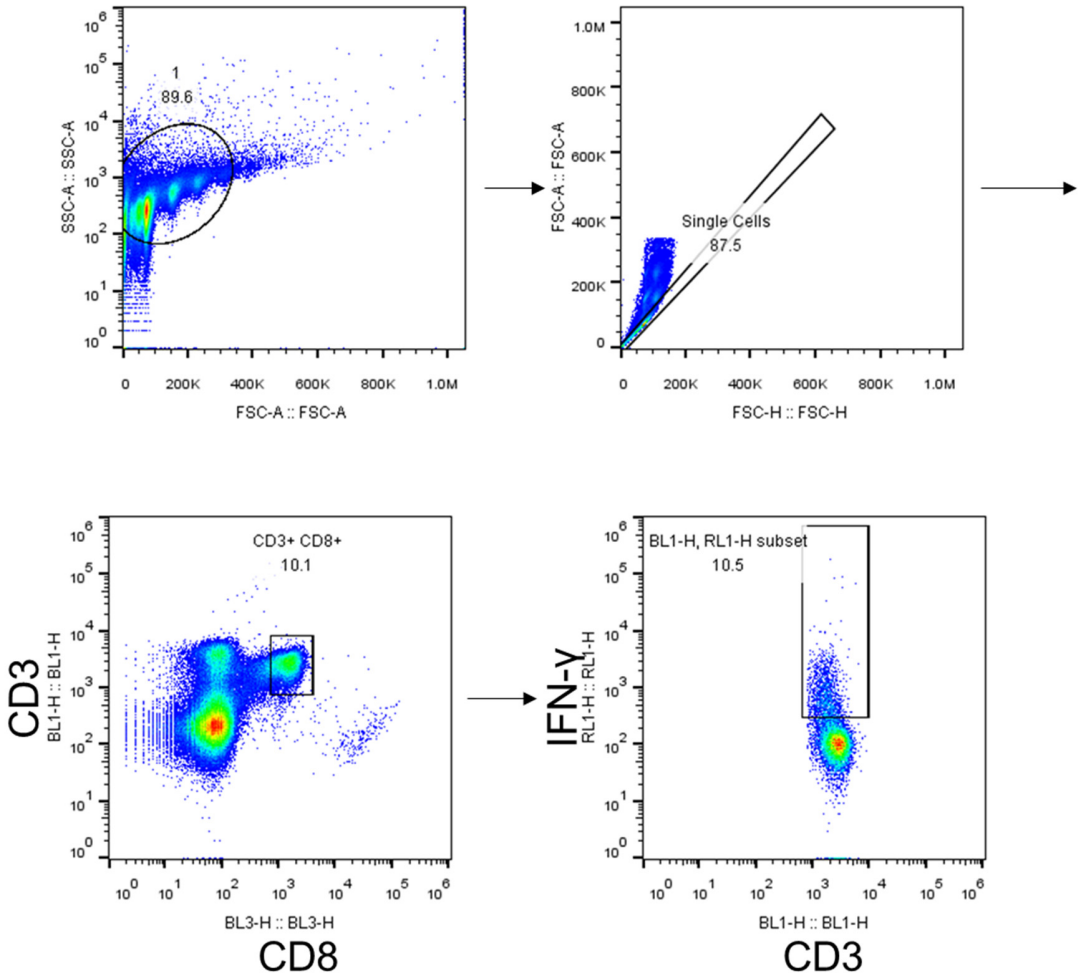

Fig. S4. Gating information for flow results of intracellular cytokine staining.

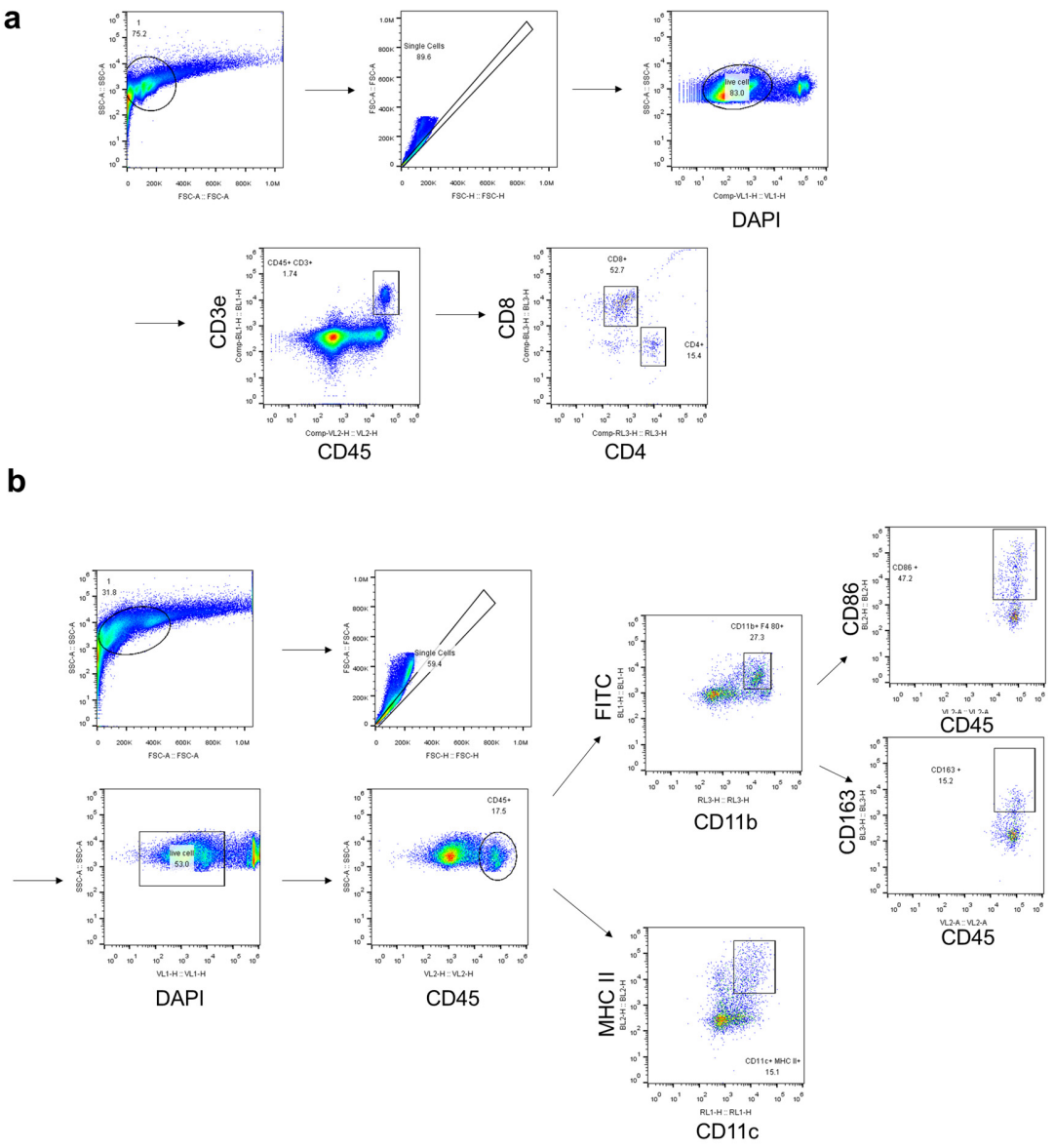

Fig. S5. Gating information for immunocellular composition experiment.

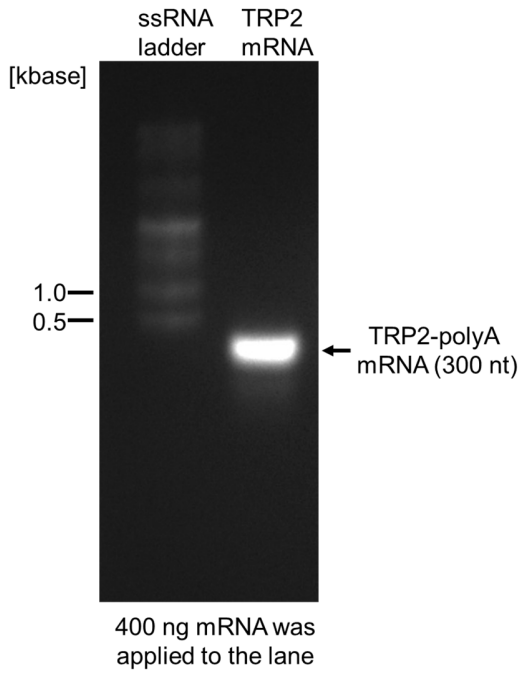

Fig. S6. Agarose gel electrophoresis of $TRP2_{180\text{-}188}$ mRNA.

Reference

1. Pittelkow, M.; Lewinsky, R.; Christensen, J.; *Synthesis*. 2002, *15*, 2195-2202.

2. Scheffer U.; Strick, A.; Ludwig, V.; Peter, S.; kalden, E.; Göbel, M.; *J. Am. Chem. Soc.* 2005, *127*, 2211-2217.