

Toll-like Receptor Activation Induces Degeneration of Human Intervertebral Discs

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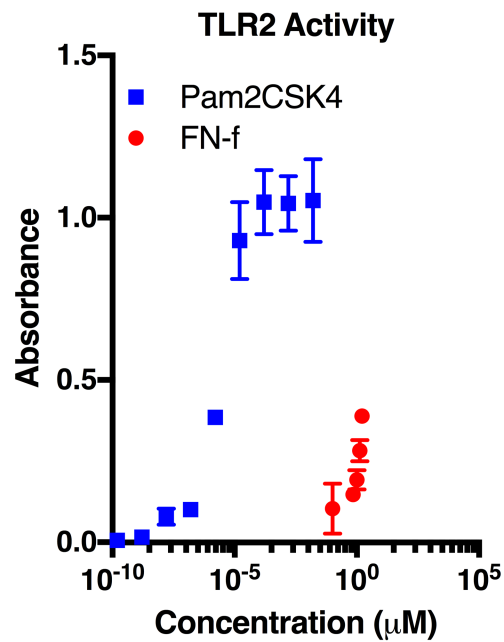
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Supplemental Figure 1



Supplemental Figure 1. TLR2 activity in response to Pam2CSK4 and 30kDa fibronectin fragments HEK 293 cells that over express TLR2 and produce SEAP following TLR2 activation were used to evaluate the activity patterns of Pam2CSK4 and 30 kDa fibronectin fragments (FN-f). TLR2 induction of SEAP activity causes the culture media to turn blue, increasing absorbance at 630nm. Cells were treated with 0.0002 pmol to 15.75 pmol Pam2CSK4 or 100 nmol to 1600 nmol FN-f for 12 hours and absorbance was measured. Data is presented as the mean \pm SEM, n=4.

Supplemental Table 1

	Donor	1	2	3	4	5	6
	Age	57	50	42	48	46	44
PBS	Disc Level	L1/2	L3/4	L3/4	L2/3	L1/2	L3/4
	Grade based on Wilke <i>et al.</i>	1	1	0	1	1	1
	Disc Height (cm)	0.84	1.17	1.13	1.08	1.01	0.97
Pam2CSK4	Disc Level	L3/4	L2/3	L2/3	L3/4	L3/4	L1/2
	Grade based on Wilke <i>et al.</i>	1	1	0	1	1	1
	Disc Height (cm)	1.01	1.9	1.12	1.1	1.01	0.86
FN-f	Disc Level	L2/3	L1/2	L4/5	L4/5	L2/3	L4/5
	Grade based on Wilke <i>et al.</i>	1	1	1	1	1	0
	Disc Height (cm)	1.07	1.2	1.24	1.19	1.00	1.01

Supplemental Table 1. Characteristics of discs injected with each treatment. Age of an individual donor is specified in years. The level of the disc injected with either PBS, Pam2CSK4, and FN-f from the lumbar region is indicated. Discs were graded based on Wilke *et al.* 2006. Disc height was determined by averaging the dorsal, ventral and midsection disc height. No differences in grade or height were found between groups prior to treatment.

Supplemental Table 2. Primers used for RT-qPCR

Gene	Primer Sequence 5' to 3'	Primer Reference
GAPDH	F: TCCCTGAGCTGAACGGGAAG R: GGAGGAGTGGGTGTCGCTGT	46
IL-1 β	F: ACAGATGAAGTGCTCCTTCCA R: GTCGGAGATTCGTAGCTGGAT	47
NGF	F: AAGTGCCGGGACCCAAAT R: TGAGTTCCAGTGCTTTGAGTCAA	48