

Additional file 1

Table S1. PCR primer sequences.

Genes		Primer sequences (5'→ 3')	References
GAPDH	Forward	TGATGACATCAAGAAGGTGGT	Bak Y, <i>et al.</i> (2014)
	Reverse	TCCTTGGAGGCCATGTAGGCC	
IL-320	Forward	TGATGTCTGAGCCTGGCAGAG	Kim MS, <i>et al.</i> (2015)
	Reverse	GAGCTCTGACAGAGAGCAGC	
CD68	Forward	GCTGGCTGTGCTTTTCTCG	Reuwer AQ, <i>et al.</i> (2011)
	Reverse	GTCACCGTGAAGGATGGCA	
E-Cadherin	Forward	GAAGGTGACAGAGCCTCTGGAT	Bak Y, <i>et al.</i> (2016)
	Reverse	ATCGGTTACCGTGATCAAAATC	
MMP-9	Forward	CGCAGACATCGTCATCCAGT	Song YS, <i>et al.</i> (2015)
	Reverse	GGATTGGCCTTGGAAGATGA	
COX-2	Forward	CAAATCCTTGCTGTTCCACCCAT	Wu XL, <i>et al.</i> (2013)
	Reverse	GTGCACTGTGTTTGGAGTGGGTTT	
IL-1β	Forward	TACATCCTCGACGGCATCTCA	Kim MS, <i>et al.</i> (2014)
	Reverse	CTACATTTGCCGAAGAGCCCT	
CCL5	Forward	GCTGTCATCCTCATTGCTAC	Bak Y, <i>et al.</i> (2014)
	Reverse	CATTTCTTCTCTGGGTGGC	
GM-CSF	Forward	AAATGTTTGACCTCCAGGAG	
	Reverse	TGATAATCTGGGTGCACAG	
CCL18	Forward	TCTATACCTCCTGGCAGATTC	Gunther C, <i>et al.</i> (2011)
	Reverse	TTTCTGGACCCACTTCTTATTG	

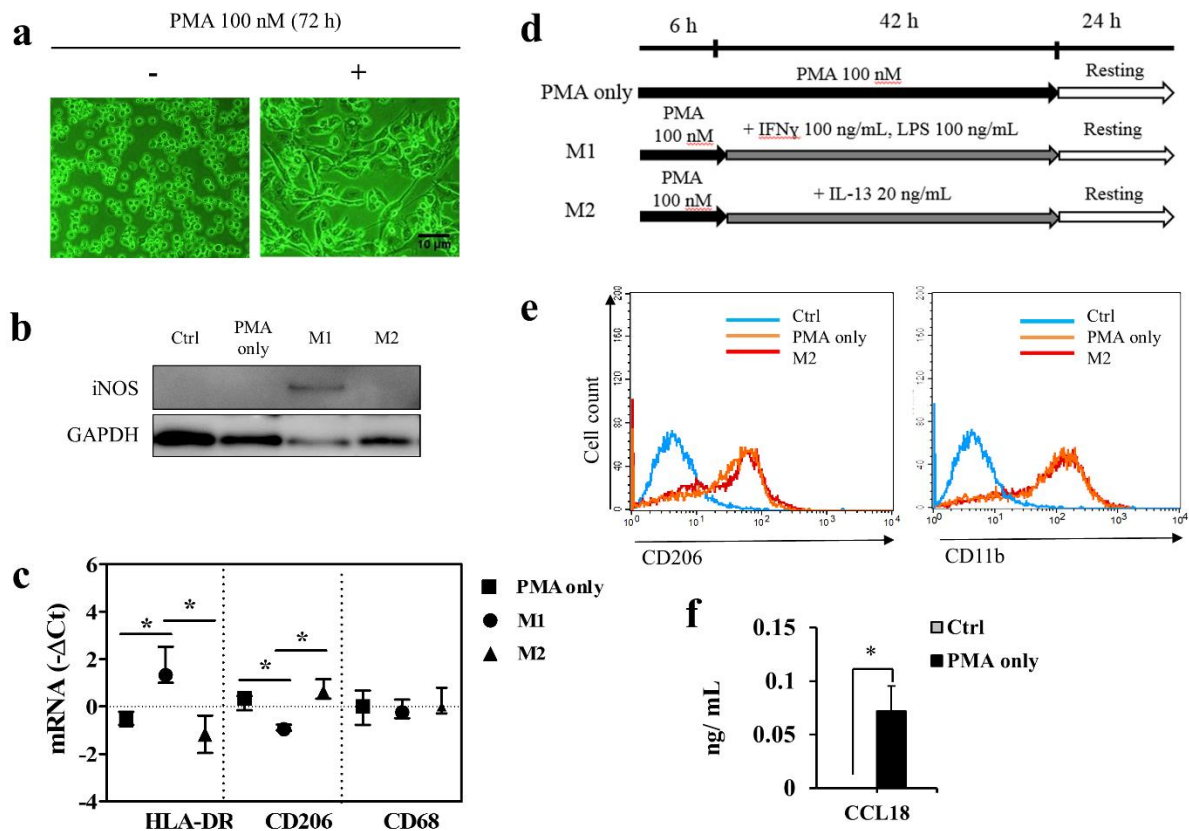


Figure S1. PMA-induced macrophages show an M2-like phenotype. a, Cellular morphological change of THP-1 cells after treatment with 100 nM PMA for 72 h. b, After treatment with 100 nM PMA for 6 h, THP-1 cells were either kept in PMA treatment conditions for an additional 42 h to differentiate into the "PMA-treated THP-1 macrophages" (labeled "PMA only"), or cultured with PMA plus 100 ng/ml LPS and 100 ng/ml IFN- γ for 42 h to become M1-polarized THP-1 macrophages (labeled "M1"), or cultured with PMA plus 20 ng/ml IL-13 for another 42 h to become M2-polarized THP-1 macrophages (labeled "M2"). Differentiated THP-1 cells were then cultured in fresh media for a further 24 h before being harvested for other experiments. c, The M1 macrophage marker iNOS was detected by western blot. d, The macrophage marker, CD11b, and M2 macrophage marker, CD206, were quantified via FACS analysis. e, Macrophage markers were quantitated by RT-qPCR. f, Secretion levels of CCL18 in THP-1 macrophages activated by PMA 100 nM were measured by ELISA and compared with those of the THP-1 control without any treatment. Results were from three independent experiments. Data are presented as the mean $\pm$ SEM and are analyzed using the Student's *t*-test: *, *p* < 0.05.

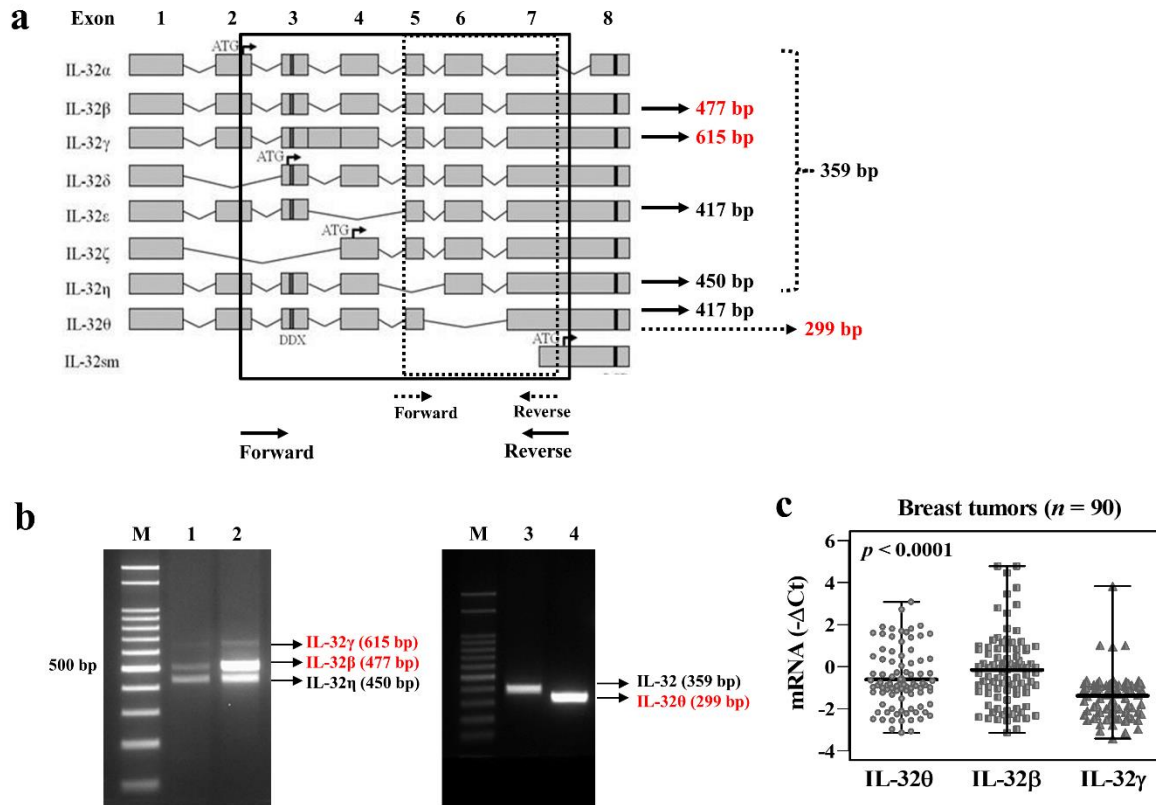


Figure S2. mRNA expression of IL-32 θ , IL-32 β , IL-32 γ in breast tumors. **a** The primers (Forward primer: 5'-GGTCCTCTCTGATGACATGA-3'; Reverse primer: 5'-TGGAAAGAGGACATGAAGAG-3') targeting the sequence from exon 2 to exon 7 were designed to detect IL-32 β (477 bp) and IL-32 γ (615 bp). Primers to detect IL-32 θ (299 bp) were used as previously described (Kim MS, *et al.*, 2015). **b** Example of mRNA expression from different IL-32 isoforms in breast tumors. **c** mRNA expression of IL-32 θ , IL-32 β , and IL-32 γ in 90 breast tumors were performed by RT-PCR and quantitated by ImageJ software. Data are presented as the median with range and are analyzed using the Kruskal–Wallis test followed by Dunn's post hoc tests: $p < 0.0001$.

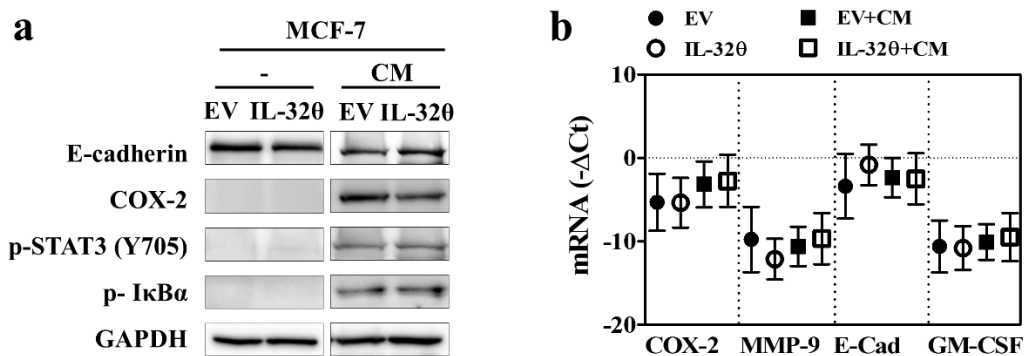


Figure S3. Effect of IL-320 on pro-malignancy and signaling factors in macrophage CM-treated MCF-7-EV and MCF-7-IL-320 cells. MCF-7 cells were transfected pcDNA3.1-6X Myc-IL-320 (MCF-7-IL-320) or pcDNA3.1(+)-6X Myc (MCF-7-EV) for 48 h then cells were treated with macrophage CM for an additional 24 h before being harvested. **a** Protein expression of COX-2, E-cadherin, phosphorylated STAT3 (Y705), and phosphorylated IκBα were measured by western blotting. **b** mRNA expression was measured by RT-qPCR. Data are presented as the mean $\pm$ SEM and are analyzed using the Student's *t*-test: *, $p < 0.05$.