Rapamycin-PLGA microparticles prevent senescence, sustain cartilage matrix production under stress and exhibit prolonged retention in mice joints

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Supplementary information

Supplementary Note 1: Curve fitting on rapamycin release profile from PLGA MPs

We observed a bi-phasic release of rapamycin from all PLGA MPs (**Figure 1d**) which is in accordance to the previously published literature.[49-51] Release profiles from different molecular weight PLGA particles were fitted using non-linear regression (least square method) two-phase exponential decay curve (**Supplementary Figure S1**). The maximum y-value (Y0) and minimum y-value (plateau) were constrained to 100 and 0 respectively. The two-phase exponential decay pattern observed in all of the PLGA MPs can be attributed to their initial burst release represented by fast exponential decay and the second phase of drug release which is slower and sustained for several days due to slower degradation of polymer chain represented by slow exponential decay. The $t_{1/2}$ values for slower exponential decay ($t_{1/2}$ slow) corresponded well to the molecular weights of PLGA with higher molecular weight PLGA exhibiting longer half-lives (**Supplementary Table S2**).

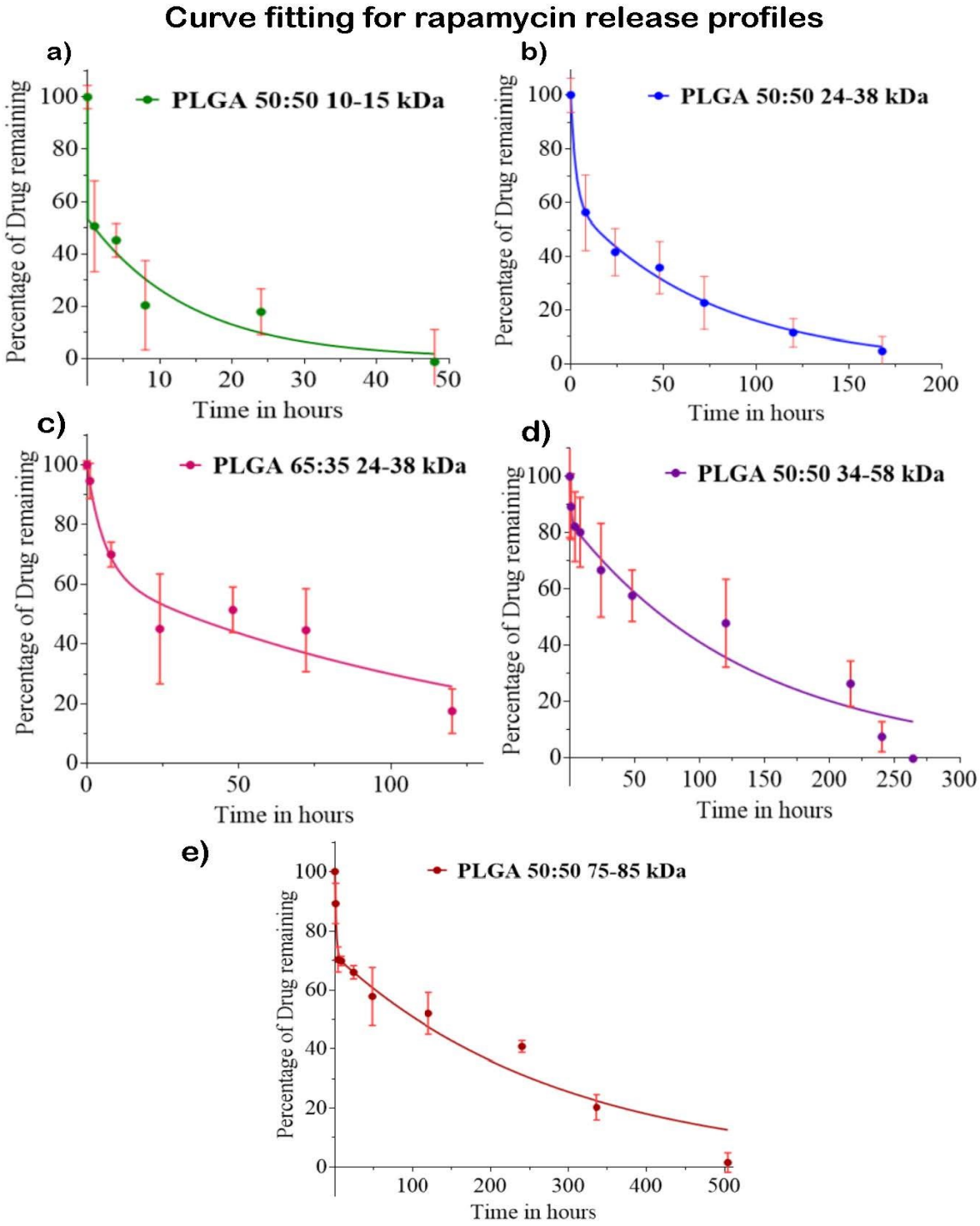

Figure S1: Curve fitting for rapamycin release from different molecular weights PLGA: Quantification of *in vitro* release profiles of rapamycin from PLGA particles synthesized from (a) PLGA 10-15 kDa (50:50) (b) PLGA 24-38 kDa (50:50) (c) PLGA 24-38 kDa (65:35) (d) PLGA 34-58 kDa (50:50) and (e) PLGA 75-85 kDa (50:50). Release profiles from different molecular weight PLGA particles were fitted using non-linear regression (least square method) two-phase exponential decay curve. Data in graphs represent the mean ± s.d. (n = 3).

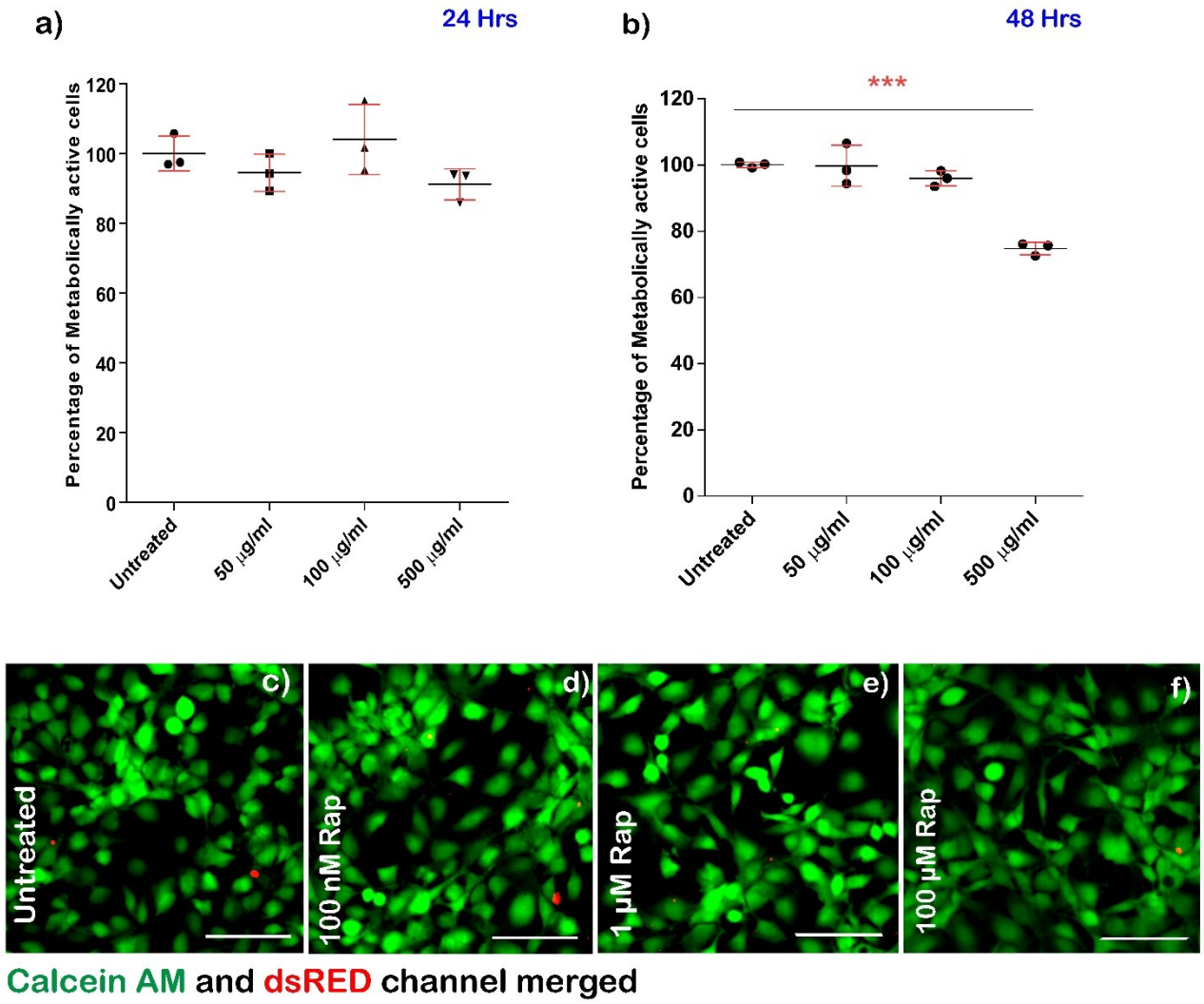

Figure S2: Cytocompatibility assays for PLGA MPs and rapamycin. Metabolic activity of chondrocytes treated with varying concentration of PLGA MPs for (a) 24 h and (b) 48 h. Images of Calcein AM (green) and Propidium Iodide (red) stained C28/I2 cells treated with (c) 0 nM, (d) 10 nM, (e) 1 µM and (f) 100 µM of Rapamycin. Scale bar 30 µm. ***p< 0.001 for 500 µg/mL PLGA MPs versus all other groups. p-value < 0.05 were considered significant. Data in graphs represent the mean ± s.d. (n = 3) and p values were determined by One-way analysis of variance (ANOVA) and Tukey's post hoc tests using GraphPad prism Software.

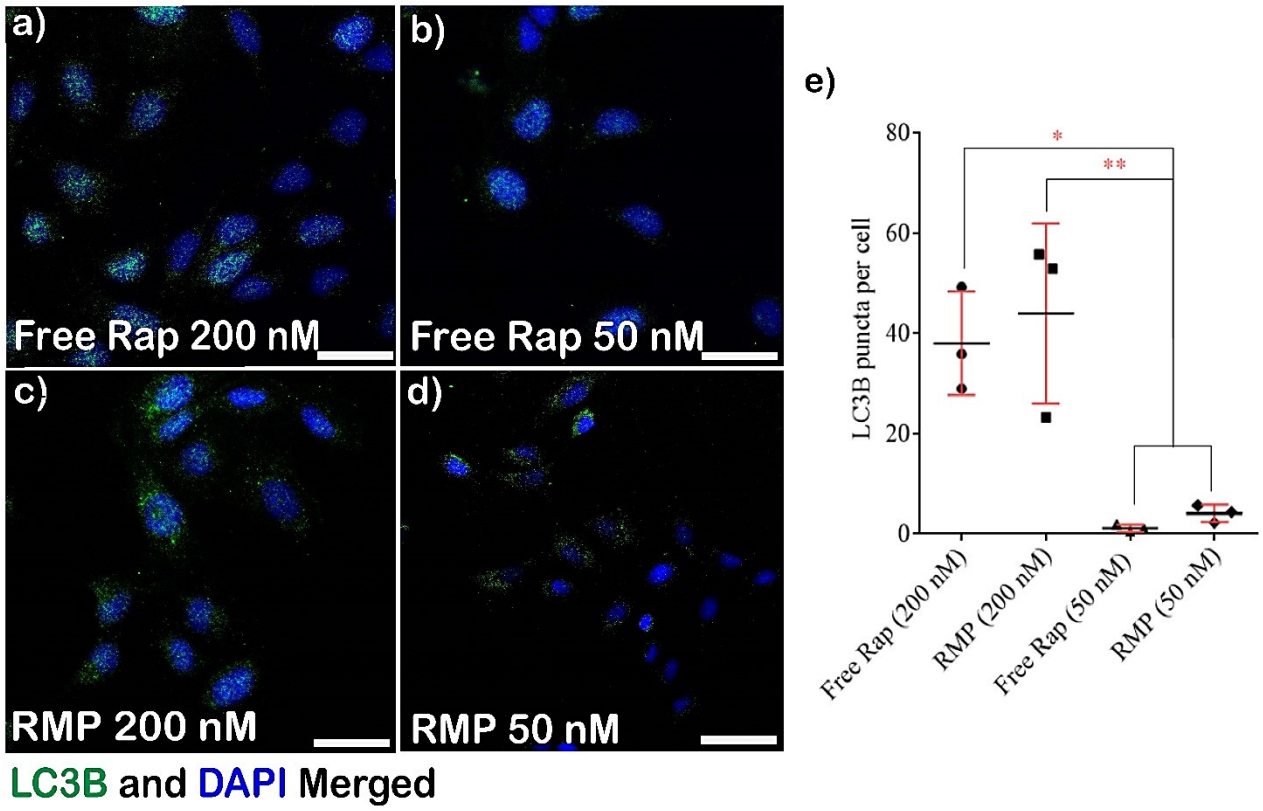

Figure S3: Rapamycin MPs induces autophagy in chondrocytes. Fluorescence microscopy images of C28/I2 cells stained with DAPI (blue) and LC3B (green) primary antibody after treatment with (a) free rapamycin 200 nM, (b) free rapamycin 50 nM (c) RMP (200 nM rapamycin) (d) RMP (50 nM rapamycin. (e) Quantification of LC3B puncta per cell using ImageJ software (n=3 per group). Data in graphs represent the mean ± s.d. and *p* values were determined One-way analysis of variance (ANOVA) test and Tukey's post hoc tests using GraphPad Prism Software. * represent $p < 0.05$ and **represent $p < 0.007$ Scale bar 20 μm.

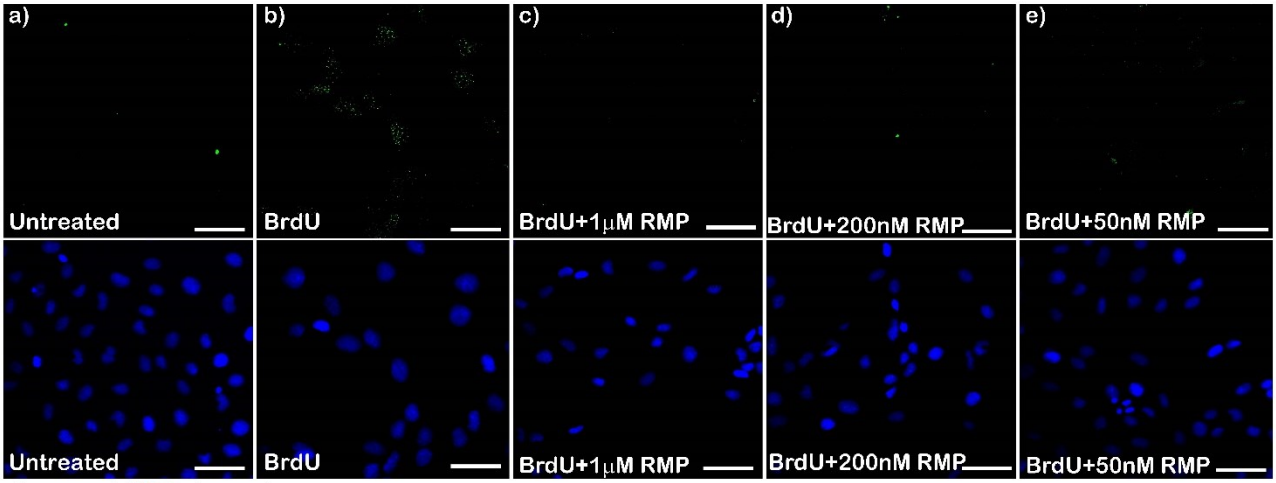

Figure S4: Rapamycin MPs reduces DNA damage in chondrocytes under genotoxic stress. Fluorescence microscopy images of C28/I2 cells stained with DAPI (blue) and γH2Ax (green) primary antibody after treatment with (a) no treatment, (b) BrdU (200 μM) treated, (c) BrdU (200 μM) along with Rapamycin MPs (1 μM), (d) BrdU (200 μM) along with Rapamycin MPs (200 nM) and (e) BrdU (200 μM) along with Rapamycin MPs (50 nM). Top Panel – FITC channel, Bottom panel – same field in DAPI channel. Scale bar 20 μm.

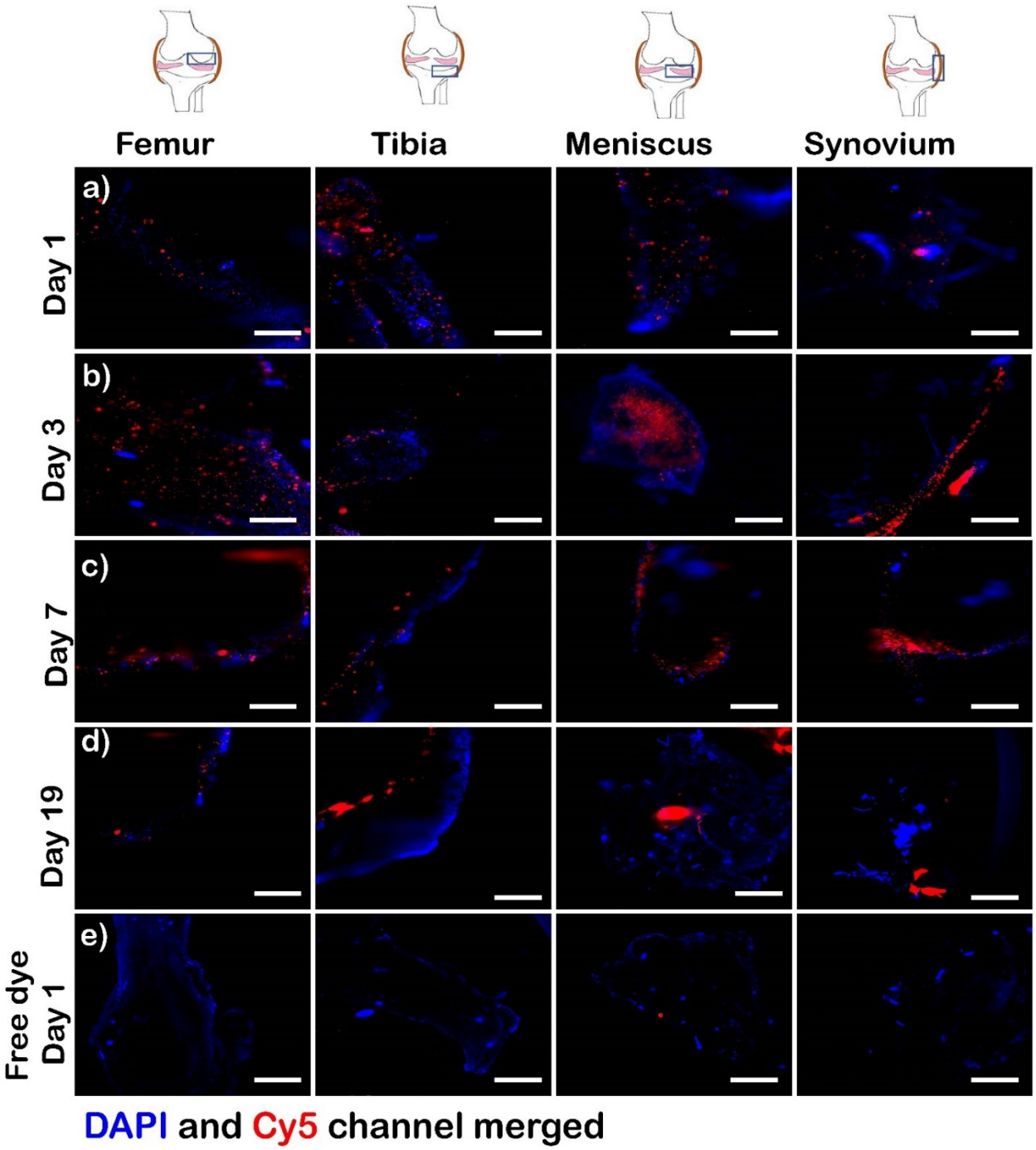

Figure S5: Cy5 labeled PLGA MPs of high molecular weight exhibit localisation to various tissues in mice knee joints. Fluorescence microscopy images of dissected mice joint tissues stained with DAPI and imaged at (a) 1 day, (b) 3 days, (c) 7 days and (d) 19 days after intra-articular injection of Cy5 labeled PLGA MPs. (e) Imaging was performed at Day 1 after injection of free Cy5 dye. Topmost insets depict the area of the joint that was imaged. **DAPI**-cells on the joint surface, **Cy5**-PLGA MPs encapsulating Cy5. Scale bar 100 μm.

Table S1: PLGA microparticles of different molecular weights and their respective sizes along with their encapsulation efficiency

Molecular weight of PLGA (Ratio of PLA: PGA)	**Size measured by DLS [nm]**	**Rapamycin encapsulation efficiency [%]**
10 kDa - 14 kDa (50:50)	1021 ± 153	63.43 ± 1.326
24 kDa - 38 kDa (50:50)	1066 ± 174.9	48.91 ± 0.930
24 kDa - 38 kDa (65:35)	946 ± 155.9	41.42 ± 1.326
34 kDa - 54 kDa (50:50)	1070 ± 183.1	41.33 ± 8.040
75 kDa - 85 kDa (50:50)	1039 ± 188	32.6 ± 3.007

Table S2: Two-phase exponential decay curve parameters for rapamycin release from PLGA microparticles of different molecular weights

Molecular weight of PLGA (Ratio of PLA: PGA)	**Correlation co-efficient (R^2)**	**Equation**	**$t_{1/2}$ (Slow) hours**
10 kDa - 14 kDa (50:50)	0.8855	$y = 46.5\ e^{-8.586*10^{7}x} + 53.5\ e^{-0.0705x}$	9.83
24 kDa - 38 kDa (50:50)	0.9396	$y = 39.75\ e^{-0.3676x} + 60.25\ e^{-0.0132x}$	52.49
24 kDa - 38 kDa (65:35)	0.8739	$y = 36.34\ e^{-0.1749x} + 46617e^{-0.0075x}$	91.79
34 kDa - 54 kDa (50:50)	0.8707	$y = 16.19\ e^{-0.9766x} + 83.81e^{-0.0071x}$	97.31
75 kDa - 85 kDa (50:50)	0.9474	$y = 28.21\ e^{-0.5847x} + 19427\ e^{-0.0034x}$	201.5