Supplemental figure legends

Fig. 1 supplemental: Dose response of cytokine induction after a single R848 injection in vivo
Mice were injected with varying doses of R848 subcutaneously (2.5 - 20 µg). Two, 4 and 8 h p.i. serum was analyzed for cytokines by ELISA. Sera of three mice were pooled for analysis.

Fig. 2 supplemental: Fractionated R848 tumor therapy is similar in efficacy to a DC vaccine combined with CpG
Mice (n = 4 to 5 per group) were inoculated with 2.5 x 10^5 CT26 colon carcinoma cells s.c. on day 0. On days 7, 11, 15 and 19 (arrows) mice were treated as follows: Controls were left untreated, the R848 group obtained 20 µg R848 four times per treatment cycle as indicated by the schedule below the graph. For DC + CpG 2 x 10^5 tumor-loaded DC per mouse were applied contralaterally followed by treatment with 200 µg CpG. Asterisks (**, p < 0.01; ***, p < 0.001) indicate significant differences to the untreated group.

Fig. 3 supplemental: Cox1/2 inhibitors do not affect tolerance induction
Bone marrow cells were stimulated for 24 h with 0.1 µg/ml R848 or left unstimulated in the presence of increasing concentrations of Cox1/2 inhibitors, washed and restimulated with 0.1 µg/ml R848. Asterisks (*, p < 0.05; **, p < 0.01; ***, p < 0.001) indicate significant differences between cells that were prestimulated with R848 (black bars) and cells that were not prestimulated (white bars). 'bdl' indicates below detection limit. Data are representative of two independent experiments.

Fig. 4 supplemental: R848 pretreatment in the ng/ml scale does not induce tolerance
Primary bone marrow cells (4 x 10^5 per well) were stimulated for 24 h with indicated concentrations (in ng/ml) of R848, washed and restimulated for 6 h with 1µg/ml R848. Cytokine levels were determined by ELISA. Secretion of cytokines was normalized to cells that were not prestimulated. Asterisks (*, p < 0.05; **, p < 0.01; ***, p < 0.001) indicate
significant differences between cells that were prestimulated with R848 (black bars) and cells that were not prestimulated (white bars).
Figure S1
Figure S2
Bourquin, Hotz et al.: R848 tolerance

**Figure S4**

R848 (ng/ml) vs. IL-12p70 (fold change)

- 0, 0.05, 0.5, 5, 50, 500 ng/ml R848

- **:** Significant difference

R848 (ng/ml) vs. IL-6 (fold change)

- 0, 0.05, 0.5, 5, 50, 500 ng/ml R848

- ***:** Significant difference
Figure S3