

Supplementary Materials for

A Novel Aldehyde Dehydrogenase 3 activator leads to adult salivary stem cell enrichment *in-vivo*

Supplementary Figure Legends

Supplementary Figure 1. Isolation of mouse salivary stem cells.

Panel A: Flow cytometry analysis of c-Kit⁺ cells isolated from mouse submandibular glands shows strong co-localization of with Sca-1 (73.8%) or CD90 (80.7%). **Panel B:** The cultured D10 salisphere show positive staining for BrdU when compared to negative staining control. **Panel C:** HE staining (left) and PAS staining (right) of D10 salisphere. **Panel D:** The cultured D10 salisphere showed positive staining for CK5, CK14, c-Kit and Sca-1. **Panel E:** Salisphere formed ductal-like structures when cultured in 3D collagen matrix. Scale bar =25 um.

Supplementary Figure 2. Alda-89 treatment did not increase the number of Aldefluor positive cells

Panel A: Aldefluor assay showed no difference in the percentage of Aldefluor positive cells between the Alda-89 (red) and the vehicle treated (blue) groups in all viable isolated SMG cells. **Panel B:** Aldefluor assay showed no difference in the percentage of Aldefluor positive cells between the Alda-89 (red) and the vehicle-treated (blue) groups in the c-Kit⁺/CD90⁺ viable SCs.

Supplementary Figure 3. Salisphere composition does not change after ALDH3 activation.

Panel A: HE staining showing no difference in the growth rate of the salispheres between the vehicle and the ALDA-89 treated groups. **Panel B:** PAS immunofluorescent staining shows similar distribution of mucopolysaccharide positive cells on culture day 10 in both groups. **Panel C:** Immunofluorescent staining showing similar distribution of CK14 positive cells (ductal marker) in the salispheres of both groups. Scale bar = 25 um.

Supplementary Figure 4. Saliva measurements after radiation in the 2 treatment groups.

Saliva production was slightly higher in the Alda-89 treated group compared to vehicle control (n=8 for each group). However, the difference was not statistically significant. Saliva measurement was

normalized to the mouse body weight at the time of measurement as well as to the pre-radiation level. $Y = \{ \text{Saliva secretion (g)} / \text{body weight (g)} \} / \{ \text{Pre RT Saliva secretion (g)} / \text{Pre RT body weight (g)} \}$.

Supplementary Table 1. Human ALDH Qpcr primers.