

Supplementary data are available at: <https://figshare.com/s/3c267f0780fe5f4219b9>

**Supplementary Figure S1: Specificity of the PhaE staining.**

(A) Part of a bud showing the general organization of the two epithelial layers: the exopinacoderm (Ex) and the choanoderm (choanocyte chambers CC) stained by the fluorescein-labeled *Phaseolus vulgaris* erythroagglutinin (PhaE) and surrounded by the mesenchymal-like mesohyl (M). (B) Focus on a choanocyte chamber (dotted red line) with a schematic representation explaining the typical organization of this structure: Mi stands for microvilli, Nu for nucleus, Fl for flagellum, PhaE stands for the PhaE staining (green) which is only visible in the cytoplasm of choanocytes (dotted pink line). (C) View of the exopinacoderm, no PhaE staining is visible in the exopinacocytes. (D) No PhaE staining is present neither in cell of the mesohyl nor in endopinacocytes lining canal (C). For all pictures: Dapi in blue, phalloidin in grey.

**Supplementary Figure S2: Comparison of the aspect of choanocyte chambers under different conditions (confocal microscopy views).**

(A) Choanocyte chambers (dotted yellow line) during the dissociation in CMFSW (Calcium Magnesium Free Sea Water) and reaggregation in NSW (Natural Sea Water) showing the reproducibility of the phenotype between choanocyte chambers (See Supplementary Table S1 for statistical analyses; For more descriptive information see Figures 3 and 4). (B) Control conditions in NSW (Natural Sea Water) related to Figures 3 and 4. (C) Choanocyte chambers during the reaggregation in NSW, after one hour (1hR): the choanocytes initiate cellular contacts (Control NSW (1hR) in B; other time-points of reaggregation are shown in Figure 4). Dapi in blue, phalloidin in grey, PhaE in green, type IV Collagen in magenta. Abbreviated as follows: 1 hour of dissociation (1hD) and 4 hours of dissociation (4hD), 1hour (1hR), 3 hours (3hR) and 24 hours (24hR) of reaggregation.

**Supplementary Figure S3: Acetylated tubulin immunostaining of the choanocyte flagella during dissociation and reaggregation.**

The choanocyte flagella (Fl) are still present (yellow) after (A) 4 hours of dissociation (4hD) and (B) 3 hours of reaggregation (3hR). Dapi in blue, phalloidin in grey, PhaE in green, Acetylated tubulin in yellow. Abbreviated as follows: 4 hours of dissociation (4hD), 3 hours of reaggregation (3hR), NSW: Natural Sea Water.

**Supplementary Figure S4: Distribution of the transcripts with CAMs conserved domain during dissociation and reaggregation.**

(A) Heatmap representing the transcripts with conserved cadherin domain (213 transcripts). Among these transcripts, 47.4% (101) are Sponge-specific. (B) Heatmap representing the transcripts with a conserved integrin domain (208 transcripts): 43.8% (91 transcripts) are Sponge-specific. (C) Heatmap representing the transcripts with an Immunoglobulin domain (506 transcripts): 32.8% (166 transcripts) are Sponge-specific. Abbreviated as follows: 1hour (1hD) and 4hours (4hD) of dissociation and 1hour (1hR), 3 hours (3hR) and 24 hours (24hR) of reaggregation. NSW: Natural Sea Water. Clusters are numbered according to Figure 8. See Supplementary Tables S2 and S7D for more detailed description about the distribution of transcripts in each phylogenetic category.

**Supplementary Figure S5: Domain composition of putative Aggregation Factors in *Oscarella lobularis*.**

Domain composition of the 5 Group 3 Aggregation Factor candidates (according to Grice *et al.*, 2017) using the InterproScan Website (<https://www.ebi.ac.uk/interpro/>). (See Supplementary Table S12 for additional information).

**Supplementary Figure S6: Validation of the antibody against *O. lobularis* type IV Collagen by peptide competition assay.**

(A) Positive Control: the type IV Collagen immunostaining is localized in the basement membrane surrounding the choanocyte chambers. (B) Competition condition: type IV Collagen immunostaining is lost after overnight incubation with the peptide corresponding to the type IV Collagen. (C) Negative control: The crumbs peptide doesn't affect the immunostaining with the antibody against the type IV Collagen: the staining is localized in the basement membrane surrounding the choanocyte chambers like in the control condition. Dapi in blue, phalloidin in grey, type IV Collagen in magenta.

**Supplementary Figure S7: Hierarchical clustering on TPM values.**

(A) Dendrogram using standardized transcripts per million (TPM) of all genes expressed during dissociation and reaggregation. (B) Inertia plot to determine the optimal number of clusters. 15 clusters showed to discriminate our data well.

**Supplementary Table S1: Estimated percentage of perturbations of the choanoderm after**

(A) 1 hour of dissociation (1hD) regarding the aspect of the microvilli collar, (B) 4 hours of dissociation (4hD) and (C) 24 hours of reaggregation (24hR) concerning the shape of the choanocyte chambers. Statistical tests were performed with Wilcoxon rank sum test with continuity correction. Table related to Figures 3, 4 and Supplementary Figure S2.

**Supplementary Table S2: Functional transcriptome annotation using groups of orthologs (eggNOG), gene ontology terms (GO), conserved domains (CDs) and analyses of gene expression with DeSeq2.** Differentially Expressed Genes (DEG) were identified with a threshold of  $p\text{value} \leq 0.05$  and with a  $\log_2$  Fold Change ( $\log_2|\text{FC}|$ ) of at least 1.5 at different times of the dissociation/reaggregation process. (A) List of transcripts with orthologs. (B) List of transcripts with GO terms. (C) List of transcripts with CDs. (D) List of transcripts without any annotations. Related to Figures 6, 7 and Supplementary Figure S4.

**Supplementary Table S3: Number of orthologs shared between species estimated with Orthofinder and Percentage of orthologs between each species and *O. lobularis*** Related to Figure 6.

**Supplementary Table S4: General information about available sponge transcriptomes** (according to Riesgo *et al.*, 2014; Ereskovsky *et al.*, 2017; Schenkelaars *et al.*, 2015; This study).

**Supplementary Table S5: Completeness of conserved domain arrangements (CDAs) estimated by the proportion of the CDAs found among the expected conserved CDAs in different metazoan species using DOGMA.** Related to Figure 6E.

**Supplementary Table S6: Differential Expressed Genes (DEG)** during (A) Dissociation process (B) dissociation and reaggregation processes and (C) reaggregation process. DEG extracted from DeSeq2 analysis with a threshold of  $p\text{-value} \leq 0.05$  and  $\log_2 |\text{Fold Change}|$  of at least 1.5 at different times of dissociation (1hD, 4hD) and reaggregation (1hR, 3hR). Related to Figure 7.

**Supplementary Table S7: Phylostratigraphy analysis.** (A) Transcriptomes and Proteomes used in Phylostratigraphy analyses. Each phylostratum represents a node. (B) Accession numbers and links of the different transcriptomes and proteomes used. (C) Phylostratigraphy analysis of the *Oscarella lobularis* transcriptome and of the differentially expressed genes during dissociation and reaggregation. (D) Number of genes with conserved CAMs domains (Cadherin, Integrin, Immunoglobulin) in each phylogenetic category with common expression profiles (see Figure 8 for cluster numbers). Related to Figure 7 and Supplementary Figure 4.

**Supplementary Table S8: GO term enrichment analysis.** (A) Enriched categories in Differential Expressed Genes (DEG) during dissociation only, reaggregation only and both (combined). (B) Enriched categories in the first 100 DEGs. (C) Enriched categories in each core clusters (see Figure 8 for cluster numbers and Supplementary Table S10 for complete list of genes included in the core cluster). Related to Figures 7 and 8. Selected GO biological processes of interest are highlighted in yellow.

**Supplementary Table S9: Identification of orthologous groups with eggNOG and BlastP searches** (A) for significantly deregulated transcripts associated with GO biological processes of interest (complete list of GO terms in Supplementary Table S8) during dissociation, reaggregation and both (combined) processes shown in Table S6, (B) for the top 100 differentially expressed genes. Related to Figure 7.

**Supplementary Table S10: List of genes included in the core clusters (1 to 15) with orthologs (eggNOG) and gene ontology terms (GO).** Related to Figure 8.

**Supplementary Table S11: Transcripts Per Million (TPM) standardized and Log2 Fold Change values for** (A) epithelial genes (see Figure 1A) and (B) Aggregation factors (AFs). Related to Figure 8 and 9.

**Supplementary Table S12: Domain predictions for putative group 3 Aggregation Factors (AFs)** using InterproScan Website (<https://www.ebi.ac.uk/interpro/>). Related to Supplementary Figure S5.

**Supplementary Table S13: Raw file including mRNA-Seq Transcript Per Million count table using Kallisto.**

**Supplementary Table S14: Mfuzz clusters stability.** For each cluster the Jaccard index was calculated to evaluate the similarity of each cluster between each run (10 runs, Run 1 was taken as reference). Related to Figure 8.

**Supplementary File S1: Translated version of the Transcriptome obtain with Transdecoder** (available at <https://github.com/TransDecoder>).